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Spectroscopic method development and validation for simultaneous estimation of neomycin sulphate and clotrimazole from bulk and pharmaceutical dosage form

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Abstract

A variety of pharmacological medications are available for the treatment of fungal infections. Spectroscopic methods are very effective for estimating such drugs. The study aimed to develop and validate a simple, sensitive, rapid, and precise UV-visible method for simultaneously estimating neomycin sulphate and clotrimazole in bulk and pharmaceutical dosage form. The λmax of neomycin sulphate and clotrimazole were found to be 244 nm and 263nm. The drug exhibits linearity within the range of 300-1800 ug/ml for neomycin sulphate and 2-12 ug/ml for clotrimazole. The minimal % RSD values indicate the method's accuracy and precision. The robustness of this method was examined by varying its wavelength. Method was found to be sensitive based on LOD and LOQ values of both drugs. It was evident from the results that the suggested method works well for the quick, precise, and accurate measurement of Neomycin sulphate and Clotrimazole by UV-Visible spectroscopy method.

Keywords: UV-Visible Spectroscopy; Clotrimazole; Neomycin Sulphate; Method Development; Validation

1. Introduction

In analytical chemistry, sophisticated technologies are used to determine the composition of substances using various analysis methods. We can provide results that are both quantitative and qualitative analysis. Analytical tools are of the utmost importance when collecting accurate and reliable analytical data. Therefore, every person in the analytical laboratory must prioritize ensuring the quality of the instrumentation [1]. The development of recognized test methods is ultimately the result of advancements in analysis techniques. Consequently, these methods were employed by quality control labs to confirm the efficacy, precision, safety, purity, and functionality of pharmaceutical preparations [2].

Analytical procedures must be initialized or revalidated before being used in regular analysis. Thorough validation is essential for pharmaceutical companies effective functioning. The ICH Q2(R1) standards specify that studies must comply with GMP and GLP requirements [3]. When creating analytical techniques for routine and stability analysis, protocols and acceptance criteria must be followed, and all validation parameters must be used [4,5].

Neomycin (Figure 1A) is an aminoglycoside family of antibiotics. Neomycin is often regarded as the most ototoxic among the aminoglycosides. Many kinds of lotions, ointments, and other medications now include neomycin sulfate, either alone or combined with polymyxin, other antibiotics, and other corticosteroids [6,7,8]. Imidazole is a five-membered heterocyclic molecule with two nitrogen atoms at the 1,3-positions and is widely used as a fundamental component for synthesizing many pharmacologically active synthetic substances [9]. The broad-spectrum antifungal drug clotrimazole, commonly known as 1-[(2-chlorophenyl)—diphenylmethyl] imidazole, is an imidazole derivative (Figure

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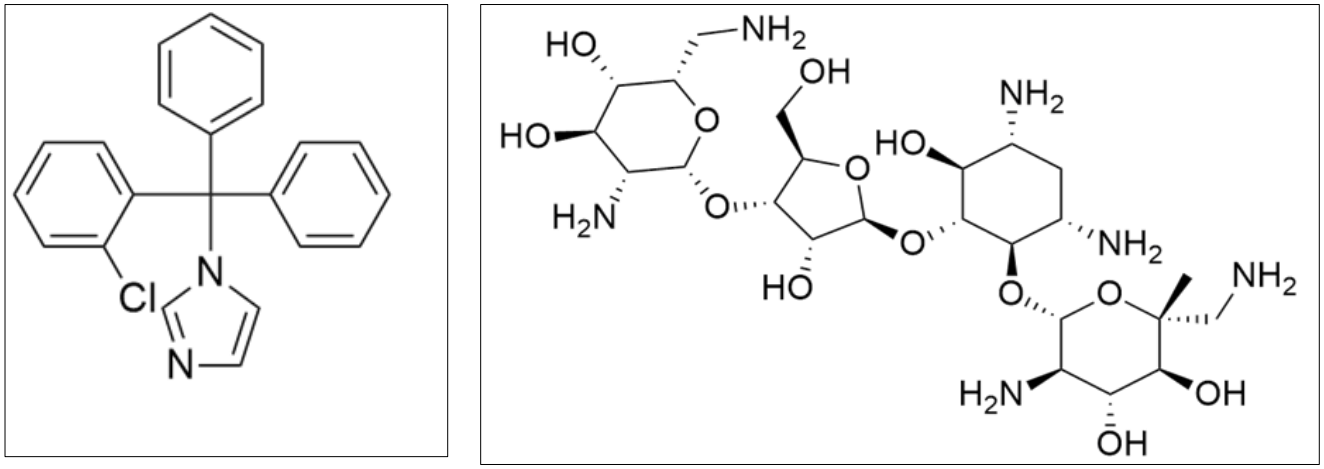
1B). By suppressing the formation of ergosterol, a component of the fungal cell membrane, this changes the permeability of the cell membrane, leading to the leakage and depletion of vital intracellular substances, ultimately leading to cell lysis [10].



(A) (B)

Figure 1 Structure of Neomycin Sulfate (A) Clotrimazole (B)

As per Google Scholar, PubMed or Science Direct database, various methods for the single and combination preparation for clotrimazole and Neomycin sulphate are described in the literature for the estimation in different dosage forms. These methods include colorimetry, RPHPLC [10-15], capillary electrophoresis with indirect UV detection [16] and HPLC using evaporative light scattering detection [17]. No reported method is available for the simultaneous estimation of clotrimazole and neomycin using UV Spectroscopy. Therefore, a simple, sensitive, precise, accurate, robust, and economical technique to quantify the combination in dosage forms using UV-visible spectroscopy, as per ICH guidelines, is needed.

2. Methods

2.1. Instruments

The study utilized an UV-Visible Spectroscopy system (UV/VIS LasanyLI-2702), Ultra Sonicator (Lifecare equipment's with 3210 model), Analytical Balance (Wear with PGB 301 model).

2.2. Chemicals and Reagents

The research employed analytical-grade materials which are essential to ensure the accuracy and reliability of the Spectroscopic analysis.

2.3. Method Development and Optimization by UV Spectrometry

Various solvents, including water, methanol, ethanol, and phosphate buffer (pH 7.4), were used to optimize the diluents for UV technique development of Neomycin sulphate and clotrimazole. Standard solutions and subsequent dilutions were prepared using optimized diluents.

2.4. Preparation of stock solution

Neomycin sulphate is found to be a pH-dependent solubility, so water does not give absorbance. For this, phosphate buffer (pH 7.4) is used as a solvent for dilution. 25 mg of Neomycin sulphate working standard were precisely measured and added to a 25 mL volumetric flask to create a standard solution. Afterwards, the pH of 7.4 phosphate buffer was added, and the mixture was shaken and subjected to sonication to dissolve the contents. The volume was then adjusted to a pH of 7.4 with phosphate buffer. For Clotrimazole, it is also found that it has pH-dependent solubility. That's why methanol and ethanol do not give absorbance. For this, phosphate buffer (pH 7.4) with ethanol is used as a solvent for dilution in a 60:40 ratio. 25 mg of Clotrimazole was added to a 25 mL volumetric flask and dissolved in ethanol and phosphate buffer (pH 7.4) (40:60). At the end, the mixture was filtered through a 0.45-micron membrane filter. The

solution was diluted with phosphate buffer to get the required concentration of the standard solution for Neomycin sulphate (300–1800 µg/ml) and Clotrimazole (2–12 µg/ml).

2.5. Determination of λ max

A UV-visible spectrophotometer operating in the 200–400 nm wavelength range was used to examine the solutions of clotrimazole and neomycin sulphate. The maximum absorption wavelength (λ max) that occurred was 244 nm for neomycin sulphate and 263 nm for clotrimazole. Figure 2 shows absorption maxima of Neomycin sulphate and clotrimazole.

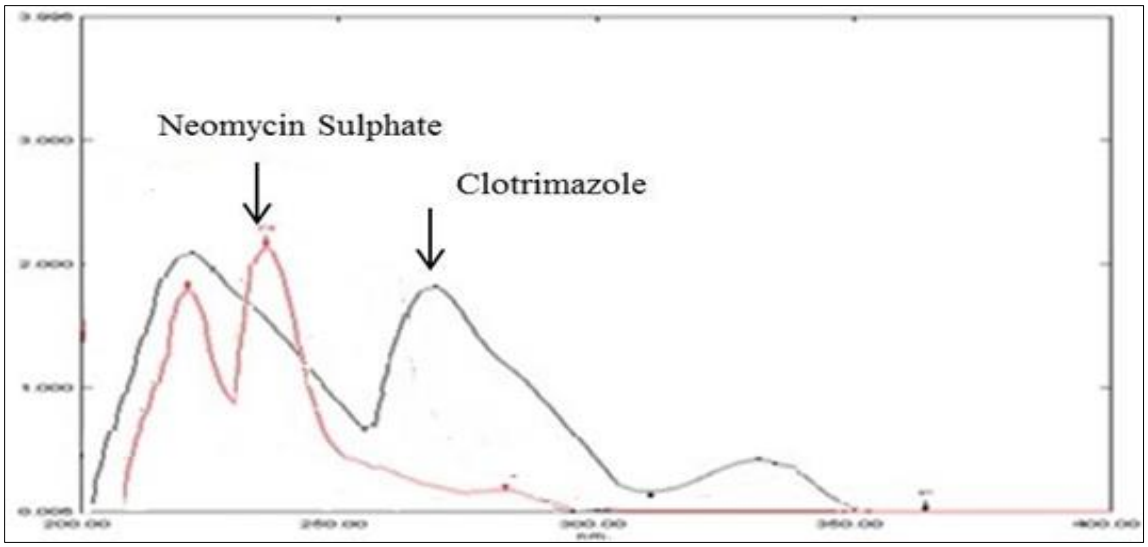


Figure 2 Absorbance maxima of Neomycin Sulphate and Clotrimazole

2.6. Method Validation for UV-Visible Method

2.6.1. Linearity

Six-point calibration curves were created to evaluate the linearity of Neomycin sulphate in the 300–1800 (µg/mL) concentration range. Furthermore, clotrimazole six-point calibration curves were produced in the 2–12 (µg/mL) concentration range.

2.6.2. Accuracy

The proposed UV technique for neomycin sulphate and clotrimazole was validated by performing recovery studies using the conventional addition approach. Standard drug concentrations in three different percentages were added to a predetermined amount of Neomycin sulphate and clotrimazole.

2.6.3. Precision

Intraday and interday precision studies of the drug were evaluated for 300, 900 and 1800 µg/ml for Neomycin sulphate and 2, 6 and 9 µg/ml for clotrimazole.

2.6.4. Robustness and Ruggedness

The robustness study was evaluated using different wavelength of both the drug.

2.6.5. LOQ and LOD

LOD and LOQ were calculated by using linearity.

3. Results

3.1. Method Validation for UV-Visible Spectroscopy

3.1.1. Linearity

It demonstrated linearity across the concentration range, according to linearity analysis. Concentration and absorbance of Neomycin sulphate and clotrimazole were reported in table 1 and 2 respectively. Linear regression equation and correlation value were reported in figure 3 and 4 for Neomycin sulphate and clotrimazole respectively.

Table 1 Concentration range and respective absorbance of Neomycin sulphate

Sr. No.	Concentration (ppm)	Absorbance
1.	300	0.278
2.	600	0.398
3.	900	0.502
4.	1200	0.626
5.	1500	0.744
6.	1800	0.844

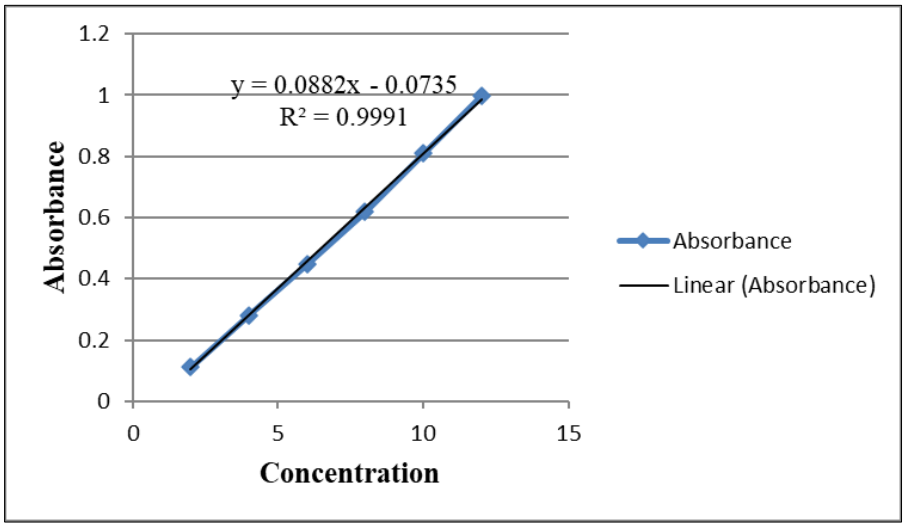


Figure 3 Standard Curve for Neomycin Sulphate

Table 2 Concentration range and respective absorbance of Clotrimazole

Serial no.	Concentration (ppm)	Absorbance
1.	2	0.112
2.	4	0.282
3.	6	0.446
4.	8	0.618
5.	10	0.809
6.	12	0.996

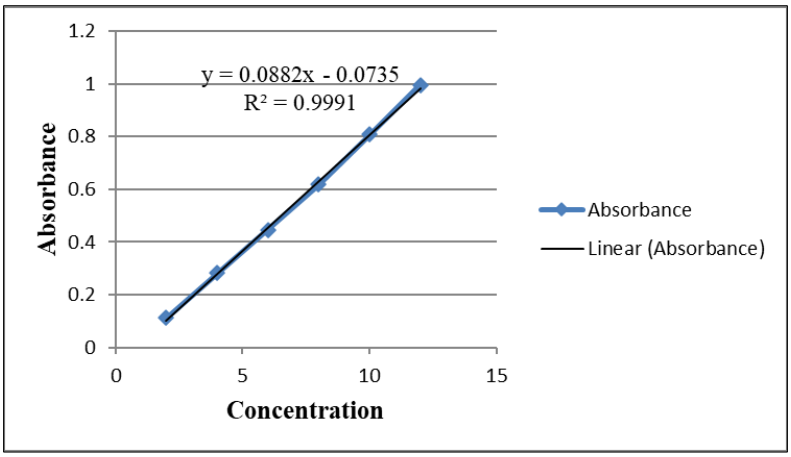


Figure 4 Standard Curve for Clotrimazole

3.1.2. Accuracy

Table 3 shows the calculated percentage recovery of additional standards. The results indicate improved mean percent recovery for the corresponding percent values.

Table 3 Evaluation data of Accuracy study of Neomycin Sulphate and Clotrimazole

Neomycin Sulphate					
Concentration(%)	**Origin level(µg/ml)**	**Amount added (PPM)**	**% Recovery**	**Mean % Recovery**	**% RSD**
80	300	240	100.00	99.66	1.527
80	300	240	98.21		
80	300	240	101.42		
100	900	900	99.20	98.92	0.220
100	900	900	99.00		
100	900	900	98.76		
120	1800	2160	100.00	99.36	0.719
120	1800	2160	99.42		
120	1800	2160	98.57		
Clotrimazole					
Concentration(%)	**Origin level(µg/ml)**	**Amount added (PPM)**	**% Recovery**	**Mean % Recovery**	**% RSD**
80	2	1.6	99.47	100.45	1.527
80	2	1.6	100.76		
80	2	1.6	101.12		
100	6	6	100.10	99.86	0.220
100	6	6	99.86		
100	6	6	99.64		
120	12	14.4	100.34	99.48	0.719
120	12	14.4	99.74		
120	12	14.4	98.37		

3.1.3. Precision

Absorbance average, % assay, and % RSD were calculated for intraday and interday precision studies (Table 4 and 5).

Table 4 Evaluation data for Intra-day and Inter-day study of Neomycin Sulphate

Intra-day	**Morning**			**Afternoon**			**Evening**		
Concentration Range (µg/ml)	**Mean**	**%Assay**	**% RSD**	**Mean**	**% Assay**	**% RSD**	**Mean**	**%Assay**	**% RSD**
300	0.278	101.03	0.2166	0.276	101.26	0.279	0.2577	101.57	0.627
900	0.398	99.23	0.7757	0.390	99.84	0.0392	0.6455	99.75	0.372
1800	0.844	98.12	0.1433	0.842	98.20	0.843	0.9142	98.64	0.142
Inter-day	**Day 1**			**Day 2**			**Day 3**		
ConcentrationRange (µg/ml)	**Mean**	**%Assay**	**% RSD**	**Mean**	**%Assay**	**% RSD**	**Mean**	**%Assay**	**% RSD**
300	0.270	101.34	1.721	0.272	101.64	1.825	0.270	101.50	0.884
900	0.389	99.43	0.639	0.394	99.76	0.145	0.391	99.12	0.523
1800	0.843	98.37	0.555	0.839	98.51	0.535	0.845	98.37	0.713

Table 5 Evaluation data for Intra-day and Inter-day study of Clotrimazole

Intra-day	**Morning**			**Afternoon**			**Evening**		
Concentration Range (µg/ml)	**Mean**	**%Assay**	**% RSD**	**Mean**	**%Assay**	**% RSD**	**Mean**	**%Assay**	**% RSD**
2	0.112	100.13	0.346	0.116	100.53	0.412	0.117	99.27	0.604
6	0.446	99.53	0.742	0.441	100.84	0.579	0.447	100.67	0.471
12	0.996	99.35	0.364	0.994	99.34	0.237	0.991	99.54	0.283
Inter-day	**Day 1**			**Day 2**			**Day 3**		
Concentration Range (µg/ml)	**Mean**	**%Assay**	**% RSD**	**Mean**	**%Assay**	**% RSD**	**Mean**	**%Assay**	**% RSD**
2	0.110	100.54	1.521	0.116	101.87	1.647	0.114	100.47	0.871
6	0.439	98.63	0.529	0.438	100.23	0.248	0.445	99.60	0.436
12	0.987	99.74	0.467	0.991	99.35	0.536	0.990	100.12	0.692

3.1.4. Robustness

The method proved robust, as shown by the %RSD values of less than 2% (Table 6).

Table 6 Evaluation data for Robustness of Neomycin Sulphate and Clotrimazole

Neomycin Sulphate			
Concentration (µg/ml)	**Wavelength**	**Absorbance**	**% RSD**
900	242	0.368	0.936
900	244	0.371	1.26
900	246	0.364	0.863
Clotrimazole			
Concentration (µg/ml)	**Wavelength**	**Absorbance**	**% RSD**
6	261	0.441	0.948
6	263	0.442	0.864
6	265	0.448	0.813

3.1.5. LOQ and LOD

For neomycin sulphate, LOD of 0.201 µg/ml and LOQ of 1.261 µg/ml were determined. The LOD for clotrimazole was 1.844 µg/ml, while the LOQ was 0.096 µg/ml.

4. Conclusion

The results demonstrate that the UV Spectroscopy method can simultaneously effectively evaluate Neomycin sulphate and clotrimazole in their respective preparations. The technique was validated according to ICH recommendations. Analysis of Neomycin sulphate and clotrimazole using this technique shows excellent consistency, accuracy, precision, linear, robust, sensitive and cost-effectiveness. Therefore, this approach can be quickly and conveniently used to measure Neomycin sulphate and clotrimazole in routine quality control simultaneously.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

References

[1] Bhagwat, V.R., Jain, C.M., Kochar, N.I. and Sonar, K.S., 2024. Review on method development and validation for different pharmaceutical dosage form. GSC Biological and Pharmaceutical Sciences, 26(3), pp.055-067.

[2] Ravisankar, P., Navya, C.N., Pravallika, D. and Sri, D.N., 2015. A review on step-by-step analytical method validation. IOSR J Pharm, 5(10), pp.7-19.

[3] Tyagi A and Dave H., 2023. Analytical method development and validation of anti-microbial formulations - a review. Int J Pharm Sci and Res, 14(9): 4344-57.

[4] Sahu, P.K., Ramisetti, N.R., Cecchi, T., Swain, S., Patro, C.S. and Panda, J., 2018. An overview of experimental designs in HPLC method development and validation. Journal of pharmaceutical and biomedical analysis, 147, pp.590-611.

[5] Jain, P.S., Chaudhari, A.J., Patel, S.A., Patel, Z.N. and Patel, D.T., 2011. Development and validation of the UV-spectrophotometric method for determination of terbinafine hydrochloride in bulk and in formulation. Pharmaceutical methods, 2(3), pp.198-202.

[6] Setiawati, H., Harmita, H. and Suryadi, H., 2020. Development and validation method for simultaneous quantification of neomycin and polymyxin B by HPLC-ELSD and comparison with microbiological method. Journal of Applied Pharmaceutical Science, 10(4), pp.129-134.

[7] Rapacz-Kmita, A., Gajek, M., Dudek, M., Kurpanik, R., Kluska, S. and Stodolak-Zych, E., 2024. Neomycin Intercalation in Montmorillonite: The Role of Ion Exchange Capacity and Process Conditions. Materials, 17(17), p.4207.

[8] Nassauer, L., Schott, J.W., Harre, J., Warnecke, A., Morgan, M., Galla, M. and Schambach, A., 2024. The caspase-inhibitor Emricasan efficiently counteracts cisplatin-and neomycin-induced cytotoxicity in cochlear cells. Journal of Molecular Medicine, pp.1-12.

[9] Orabi, M.D., Khalil, W., Alzobar, K. and Merza, J., 2024. Synthesis and Characterization of the New Imidazole-Derivative Salts' Nanoparticles and Studying of Its Biological Activity. Nanosistemi, Nanomateriali, Nanotehnologii, 22(2).

[10] Kalshetti, S. and Rao, K.P., 2021. Development and validation of HPLC method for the quantification of clotrimazole in a gelatin film formulation. Future Journal of Pharmaceutical Sciences, 7, pp.1-9.

[11] Francisco, F.L., Saviano, A.M., Pinto, T.D.J.A. and Lourenço, F.R., 2014. Development, optimization and validation of a rapid colorimetric microplate bioassay for Neomycin sulphatein pharmaceutical drug products. Journal of microbiological methods, 103, pp.104-111.

[12] Jhade, D., Dwivedi, S. and Jain, N.K., 2021. Stability indicating method development and validation of Beclomethasone dipropionate and Neomycin sulphate Ointment using HPLC with microbial assay. International Journal of Pharmacy and Life Sciences, 12(8).

[13] Ali, M.A., Amin, M.A. and Abd Halim, E.M., 2022. Green RP-HPLC Stability-Indicating Assay Method for Neomycin sulphatein the Veterinary Formulation. Egyptian Journal of Chemistry, 65(11), pp.155-162.

[14] Permata, Y.M., Bachri, M., Reveny, J. and Sibuea, F.M., 2019. Formulation and Quantitative Analysis of Betamethasone Valerate and Neomycin sulphateCream by High Performance Liquid Chromatography and Spectrophotometry. Open Access Macedonian Journal of Medical Sciences, 7(22), p.3841.

[15] Sathwik M, Raghav PV, Kumari AS, Rahaman SA. 2018. Development and Validation of New Analytical Method for the Estimation of Beclomethasone Dipropionate, Clotrimazole and Neomycin Sulphate in Bulk and Pharmaceutical Dosage Forms; 8(3): 88-91

[16] Srisom, P., Liawruangrath, B., Liawruangrath, S., Slater, J.M. and Wangkarn, S., 2007. Simultaneous determination of Neomycin sulphateand polymyxin B sulfate by capillary electrophoresis with indirect UV detection. Journal of pharmaceutical and biomedical analysis, 43(3), pp.1013-1018.

[17] Clarot, I., Regazzeti, A., Auzeil, N., Laadani, F., Citton, M., Netter, P. and Nicolas, A., 2005. Analysis of Neomycin sulphateand framycetin sulfate by high-performance liquid chromatography using evaporative light scattering detection. Journal of Chromatography A, 1087(1-2), pp.236-244.

[18] ICH (2005) ICH Harmonised Tripartite Guideline Q2(R1) Validation of Analytical Procedures: Text and Methodology. ICH Harmonized Guideline, pp 1–17.

[19] Aminu, N., Chan, S.Y., Khan, N.H. and Toh, S.M., 2018. Concurrent determination of triclosan and flurbiprofen by high-performance liquid chromatography in simulated saliva and its application in dental nanogel formulation. Acta chromatographica, 30(4), pp.219-224.

[20] Aminu, N., Chan, S.Y., Khan, N.H., Farhan, A.B., Umar, M.N. and Toh, S.M., 2019. A simple stability-indicating HPLC method for simultaneous analysis of paracetamol and caffeine and its application to determinations in fixed-dose combination tablet dosage form. Acta Chromatographica, 31(2), pp.85-91.

[21] Gupta, V., Jain, A.D.K.J., Gill, N.S. and Guptan, K., 2012. Development and validation of HPLC method-a review. International research journal of pharmaceutical and applied sciences, 2(4), pp.17-25.

[22] Murugan, S., Elayaraja, A., Chandrakala, K., Ramaiah, P. and Vulchi, C., 2013. A review on method development and validation by using HPLC. International journal of novel trends in pharmaceutical sciences, 3(4), pp.78-81.