Simultaneous Estimation and Quantification of Paracetamol, Ibuprofen and Caffeine by RP-HPLC Method in Pharmaceutical Dosage

Pankaj Deora[1], Gurunath V. Pandit[2], Shailendra V. Dalvi[3], Serra C. Dias[4]
[1]*Research Student, Quantitative, Quality Chemistry, Chikitsak Samuha's Patkar Varde College, Goregaon west, Mumbai-400061, Maharashtra, India*
[2]*Joint Secretary, Quantitative Quality Chemistry, Chikitsak Samuha's Patkar Varde College, Goregaon west, Mumbai-400061, Maharashtra, India*
[3]*Course Coordinator, Quantitative Quality Chemistry, Chikitsak Samuha's Patkar Varde College, Goregaon west, Mumbai-400061, Maharashtra, India*
[4]*Msc Student, Quantitative Quality Chemistry, Chikitsak Samuha's Patkar Varde College, Goregaon west, Mumbai-400061, Maharashtra, India*

Abstract- A fast and precise HPLC method has been established for simultaneous detection of Paracetamol, Ibuprofen and caffeine. Chromatographic profiling of the three pharmaceuticals was performed on Shimadzu Prominence LC20A System using LiChrospher® 60 RP – select B of dimension 250 mm length, 4.6 mm ID and 5.0µ Column. The instrument was controlled and data were acquired using Lab Solutions software. A gradient HPLC method was employed using two mobile phases. The Aqueous phase (Mobile phase A) was prepared by dissolving 0.5 g of octane sulfonic acid in 1000 mL Milli-Q water and orthophosphoric acid was used to adjust the pH to 3.0. Methanol was used as the organic phase (Mobile phase B). The system was run at constant flow rate of 1.0 mL/min. Separation was completed within 25 minutes. The method underwent comprehensive validation assessing parameters such as specificity, precision, linearity, accuracy and robustness. Each parameter was found to fall within the limits recommended by ICH guidelines.

Keywords: Paracetamol, Ibuprofen, Caffeine, HPLC, ICH guidelines.

I.INTRODUCTION

Drug combination therapy involves using two or more drugs in fixed dosage forms to treat the disease effectively. [1] Combined pharmaceutical dosage are being developed and used most frequently to improve results for fast recovery. Scientifically proven that multicomponent treatment is more effective than monocomponent treatment because it lowers the dosage, causes fewer side effects and it can be used as personalized treatment where doctor can mix and match drugs based on patient's condition or generic factor etc. [2]

Paracetamol (acetaminophen) is one of the most widely used analgesics.[3] Paracetamol[Fig.1] chemically, 4-hydroxyacetanilide/N-(4-hydroxyphenyl) acetamide, molecular formula $C_8H_9NO_2$, molecular weight 151.2g/mol with 4 gm daily divided dose. It is also antipyretic. [4] It is indicated for the relief of minor aches and pains, cold and flu remedies. Caffeine [Fig.2], chemically 3,7-dihydro-1,3,7-trimethyl-1H-purine-2,6-dione/1,3,7-trimethyl-3,7-dihydro-1H-purine -2,6-dione, molecular formula $C_8H_{10}N_4O_2$, Molecular weight 194.2g/mol with 300 to 600 mg dose. As analgesic boosting effect of caffeine same as paracetamol so that in 1984 it is proven that caffeine could lower the dose of paracetamol by about 40%.[5] Ibuprofen is NSAID (Non-steroidal anti-inflammatory drug) which works as a blocker of enzymes which causes paining, swelling and fever.[6] Ibuprofen [Fig.3], chemically,(RS)-2- (4- isobutylphenyl) propionic acid /(2RS)-2-(4-(2-Methyl propyl)phenyl) propionic acid , molecular formula $C_{13}H_{18}O_2$, molecular weight 206.3g/mol with 600 mg to 1.2 g daily divided dose. Paracetamol, Ibuprofen and caffeine are main active ingredients widely used in multicomponent pharmaceuticals. Paracetamol is common pain reliever and fever reducer. Caffeine is

natural stimulant which helps to wake up the brain, making people feel less tired and more alert, when included in medicine; it can boost effectiveness of other ingredients. [7]In India, the available combinations of analgesics with caffeine include Ibuprofen+ paracetamol+ caffeine and Paracetamol+ caffeine, Since, ibuprofen is more effective than paracetamol, the first combination was chosen for study. As a results, 325 mg paracetamol, 25 mg of caffeine and 400 mg of Ibuprofen used in various applications like Moderate to severe pain, Post surgical pain, Pain due to inflammation, migraine relief and fever reduction.[8] "Ensuring quality control of multicomponent pharmaceutical products demands quick and accurate techniques". As such, this paper presents a reliable, straightforward, and accurate method that employ High-performance Liquid chromatography (HPLC) for comprehensive analysis.

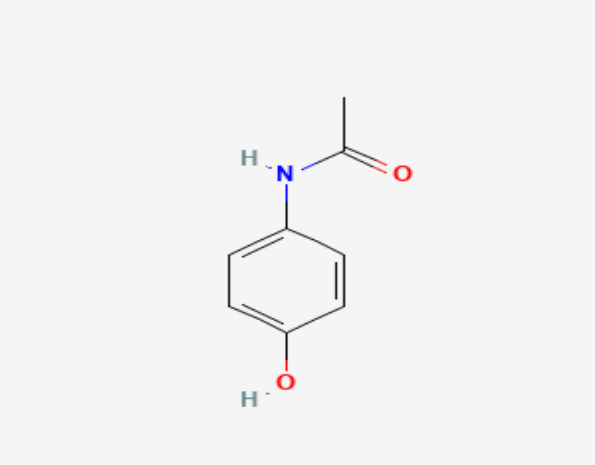

Fig. 1. Paracetamol

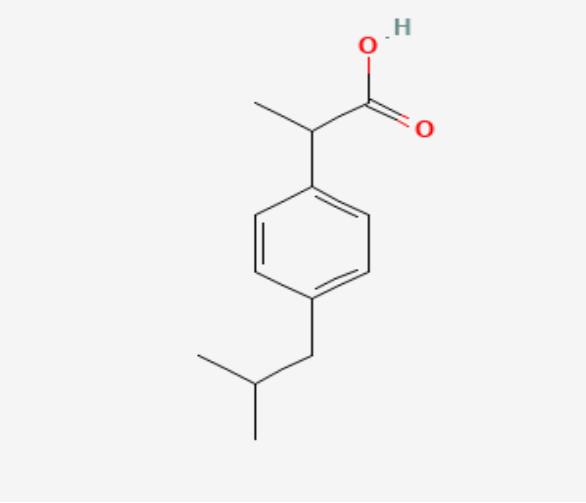

Fig. 2. Ibuprofen

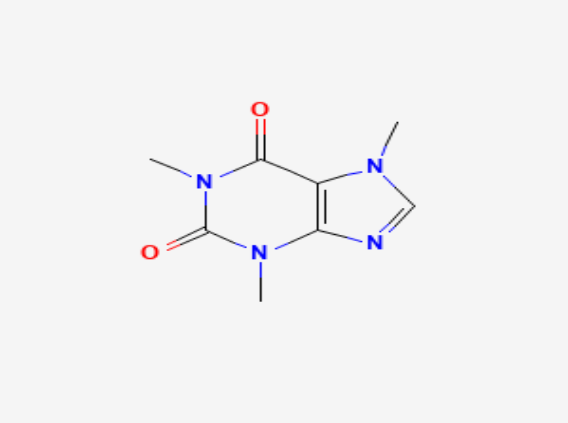

Fig.3 Caffeine

II.RESEARCH METHODOLOGY

IP reference standards for Paracetamol, Ibuprofen and Caffeine were procured from Indian Pharmacopeia Commission (Ghaziabad, Uttar Pradesh). Fixed-dose combination tablet Imol plus (Zydus Healthcare LTD) containing 325 mg of paracetamol, 25 mg of caffeine and 400 mg of Ibuprofen was purchased from a local market in Goregaon west, Maharashtra. Methanol, 1-octane sulphonic acid sodium salt and orthophosphoric acid used were HPLC grade, purchased from Merck chemicals. All dilutions were performed in standard volumetric flasks.

Instrumentation and Chromatographic Conditions:
Chromatographic analysis was conducted using a Shimadzu high-performance liquid chromatograph (HPLC) system, consisting of an LC20AD pump, a 20µL injection loop, and a Shimadzu SPD20A detector. A double beam spectrophotometer was utilized for scanning and selecting the detection wavelength. Chromatograms and data were recorded with Lab solution Software. The analysis carried out on a LiChrospher® 60 RP -Select B (5µ, 250 mm, 4.6 mm) column. The mobile phase was 0.05% 1- octane sulphonic acid sodium salt (pH 3.0 with orthophosphoric acid) and Methanol in gradient mode with column oven temperature maintained at 40°C and elution monitored by UV detector at 222 nm.
Sample injections of 20 µL were made, with samples dissolved in diluent consisting the mixture of Mili-Q water and Methanol in the ratio 1:1(pH adjusted 3.0 with orthophosphoric acid). The diluent formulation

was altered during development of bio-analytical procedure. [9]

Table No.1 Gradient Programme for Optimized HPLC Method for elution

Preparation of solution:

Time (Mins)	Composition	
	MP A (%)	MP B(%)
0.01	60	40
6.00	60	40
8.00	30	70
20.00	30	70
22.00	60	40
25.00	60	40

Paracetamol Standard stock solution:

Accurately weighed 32.5 mg of Paracetamol was transferred to 100 ml volumetric flask containing the diluent, sonicated to dissolve, then made up to the volume with diluent and followed by complete mixing.

Caffeine Standard stock solution:

Precisely Weighed 10 mg of caffeine was transferred to 20 ml volumetric flask containing the diluent, sonicated to ensure dissolution, adjusted to the volume with diluent and mixed well.

Ibuprofen standard stock solution:

Weighed accurately 40 mg of Ibuprofen was transferred into 100 ml volumetric flask containing sufficient diluent, sonicated to dissolve, then made up to the volume with diluent and mixed well.

Standard Preparation Mixture A:

From the prepared Stock solutions, pipette 20 ml of Paracetamol standard, 1 ml of Caffeine standard, and 20 ml of Ibuprofen standard and transferred into 50 ml volumetric flask. The volume adjusted with diluent. Mixed well.

Standard Preparation Mixture B:

From the prepared Stock solutions, pipette 20 ml of Paracetamol standard, 1 ml of Caffeine standard, and 20 ml of Ibuprofen standard and transferred into 50 ml volumetric flask. The volume adjusted with diluent and mixed well.

Sample Preparation:

The average weight of 20 tablets was determined by weighing them individually. A quantity of powdered tablet equivalent to approximately 0.815 gm, containing 325 mg of paracetamol, 25 mg of caffeine and 400 mg of ibuprofen, was weighed accurately and transferred into 100 mL volumetric flask. About 50mL of diluent was added. The flask was then sonicated for 20 minutes, with intermittent shaking, to ensure complete dissolution of the powder. Once the solution had cooled, the volume was adjusted to 100ml with diluent. The resulting solution is filtered and 2.0 mL of filtered solution was transferred into 50 mL flask, the volume was made up with diluent and mix thoroughly.

Method Validation:

The proposed method was validated as per ICH guidelines [10] covering aspects such as specificity, linearity, accuracy, precision, robustness and solution stability. [11]

Linearity

Linearity of the method was evaluated by injecting seven different concentrations of drug, prepared in the diluent, in triplicate into HPLC system while keeping a constant injection volume. The calibration curve was generated by plotting response factor against concentration of each drug. [12] The peak shapes were illustrated in Fig.7. A linear relationship was observed across the concentration ranges specified in Table 2.0. The corresponding calibration graphs for each drug were presented in Fig.4, 5, 6.

Table 2.0: Analysis performance data for paracetamol, caffeine and Ibuprofen

	Paracetamol	Caffeine	Ibuprofen
Linear working range	65 to 195 µg/mL	65 to 195 µg/mL	65 to 195 µg/mL
Slope	8658.58539	5202.16150	47496.19750
Intercept	9979.89060	4431.90602	21795.11821
Correlation coefficient	0.9999	0.9976	0.9999

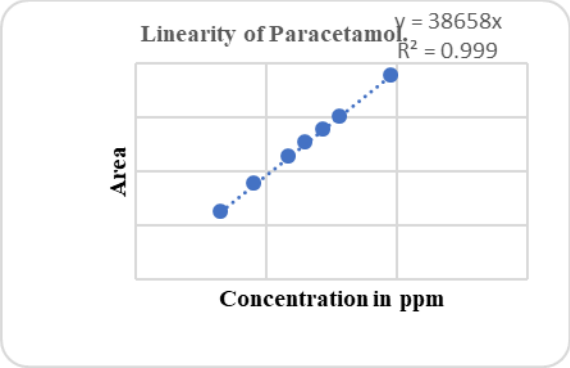

Fig. 4. Linearity plot for Paracetamol

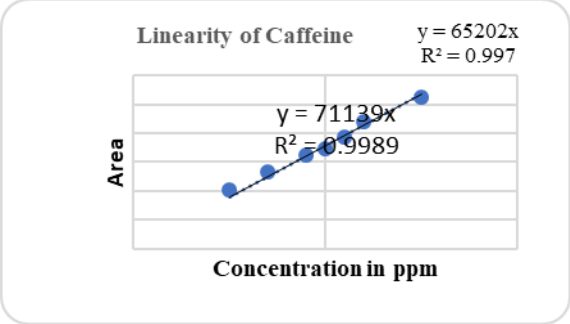

Fig. 5. Linearity plot for Caffeine

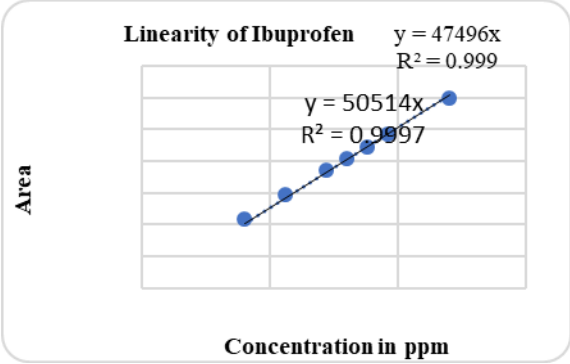

Fig. 6. Linearity plot for Ibuprofen

Limit of Detection and Limit of Quantification:

To determine limit of detection and the limit of quantification, standard solutions from low concentrations were injected using the developed method. The results were summarized in Table 3.0.

Table No 3.0 LOD and LOQ determination

Parameter	Paracetamol	Caffeine	Ibuprofen
Concentration Range (µg/ml)	65 to 195	5.02 to 15.05	80.08 to 240.24
LOD(µg/ml)	0.43	0.87	0.90
LOQ(µg/ml)	1.23	2.62	2.74

Accuracy:

Accuracy indicates how closely results obtained by the method match the true or accepted value. It is represented as the percentage of analytes recovered after adding a known amount to the sample. In this study, recovery was evaluated using standard addition method by spiking the sample at three concentrations level: 50%,100% and 150% of the intended concentration.[13] Accuracy was assessed in terms of percent recovery, as shown in Table No 4.0.

Table 4.0 Accuracy of Paracetamol, Caffeine, and Ibuprofen

Accuracy Level (%)		Paracetamol	Caffeine	buprofen
50	Mean	104.6	101.0	102.1
100		108.4	101.8	104.3
150		103.6	101.0	107.0
50	SD	0.058	0.300	0.153
100		0.058	0.100	0.306
150		0.058	0.400	0.300
50	RSD	0.1	0.3	0.1
100		0.1	0.1	0.3
150		0.1	0.4	0.3

Precision

Precision in an analytical procedure refers to the degree of consistency between repeated measurements of the same homogeneous sample under specified conditions. [14], [15]. The method's precision was assessed by conducting both interday and intraday variation studies. For both studies, six replicate injections of mixed standard solution and sample solution were performed and the percentage relative standard deviation (RSD) was calculated. Based on the obtained data, the developed HPLC method was found to be precise. The details of the results were presented in Table No 5.0 and Table No 6.0.

Table No 5.0 Assay results of spotting repeatability /Instrument precision

Sample	Paracetamol	Caffeine	Ibuprofen
As is sample solution-1	101.54	101.59	103.11
As is sample solution-2	102.44	98.69	103.88
As is sample solution-3	102.62	101.75	103.80

As is sample solution-4	101.33	100.48	102.45
As is sample solution-5	102.61	101.69	103.90
As is sample solution-6	102.85	102.18	104.80
Mean	102.37	101.06	103.66
STD DEV	0.63	1.29	0.80
% RSD	0.62	1.3	0.8

Table No 6.0 Assay results of Intra-assay/within day precision

Sample	Paracetamol	Caffeine	Ibuprofen
As is sample solution-1	101.54	101.59	103.11
As is sample solution-2	102.44	98.69	103.88
As is sample solution-3	102.62	101.75	103.80
As is sample solution-4	101.33	100.48	102.45
As is sample solution-5	102.61	101.69	103.90
As is sample solution-6	102.85	102.18	104.80
Mean	102.37	101.06	103.66
STD DEV	0.63	1.29	0.80
% RSD	0.62	1.3	0.8

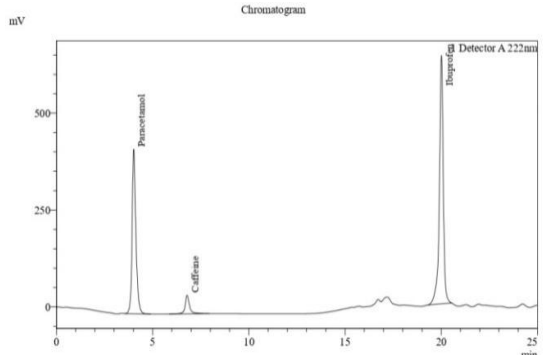

Fig.7. Representative Chromatogram of Standard for Paracetamol, Caffeine, and Ibuprofen

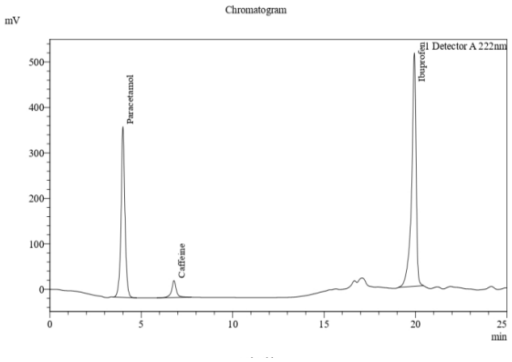

Fig.8. Representative Chromatogram of Sample for Paracetamol, Caffeine, and Ibuprofen

Stability Studies:

During method development, it is essential to assess the stability of solutions to verify the reliability of the analytical procedure. This evaluation should be part of the complete method validation process before the registration of drug product. [16]

The stability tests were designed to examine all potential conditions the sample may experience, from collection through to analysis. The stability of the drug spiked into solution was examined under short-term conditions at room temperature with sample taken at 0, 2, 4, 8,12 and 24 hours. The stability of the analytes in the solution was verified for up to 24 hrs at room temperature.

Robustness:

Robustness is an indication of the reliability of an analytical method under a variety of normal but variable conditions. [10], [17]

To assess the robustness of the proposed method, variations made to the detection wavelength (± 2 nm,220 nm and 240 nm) and the flow rate was altered between 0.9 ml /min and 1.1 mL/min. The results revealed no substantial changes in peak resolution or retention time for paracetamol, caffeine and ibuprofen under the tested conditions. These results affirm that the developed method successfully separates the three active ingredients, highlighting its robustness.

III.RESULTS AND DISCUSSION

Pharmaceutical analysis plays an essential role in the regulatory certification of marketed drugs and their formulations. Developing new analytical methods for

drug analysis is crucial for understanding pharmacokinetics, toxicology and biological consequences. The proposed method for pharmaceutical analysis occupies a pivotal role in statutory certification of marketed drugs and their formulations. Significantly, the purpose of new analytical method development for the determination of drugs in pharmaceutical dosage forms can make, it feasible to study pharmacokinetics, toxicological and biological. The proposed method is innovative, straightforward, reliable, precise, economical and reproducible, making it ideal for estimating paracetamol, caffeine and ibuprofen in their combined dosage forms, unaffected by the presence of excipients. Validation of the developed method was carried out as per the ICH guidelines, confirming the reliability. Each component shows excellent linearity at 222 nm, with correlation coefficients (R^2) of 0.999 for paracetamol, 0.9976 for caffeine and 0.9999 for Ibuprofen. Based on the linearity curve, the LOD and LOQ values were estimated and documented in Table No 3.0. Accuracy was evaluated based on % recovery, yielding results of 105.5% for paracetamol, 101.3% for caffeine and 104.4% for Ibuprofen. Precision was examined by conducting both method precision and intermediate precision, with RSD values remaining within acceptable limits. The robustness study involved minor intentional changes to chromatographic conditions, in the absence of noticeable differences. Consequently, this method is applicable for future use in the combined estimation of paracetamol, caffeine and ibuprofen in bulk pharmaceutical formulation.

IV.CONCLUSION

The proposed gradient reverse-phase HPLC method is designed for the simultaneous evaluation of paracetamol, caffeine and ibuprofen. The LiChrospher® 60 RP-Select B column is optimal for ensuring system suitability and robustness. The validated method is suitable for routine analysis and quality control of paracetamol, caffeine and ibuprofen in bulk therapeutic formulations.

V.ACKNOWLEDGEMENTS

The authors sincerely appreciate Quantitative Quality Chemistry, Chikitsak Samuha's Patkar-varde college, Goregaon (west), Mumbai, for providing the laboratory with the necessary equipment that contributed to the success of this research study.

Conflict of interest Declaration

There are no conflicts of interest to report.

REFERENCE

[1] Nafiu Aminu, Siok-Yee Chan1, Nasir Hayat Khan, Ahmed Bassam Farhan, Muhammad Nura Umar and Seok-Ming Toh (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 Chromatographia 31 (2019)2, 85-91.

[2] Hoang Vu Dang, Huong Truong Thi Thu, Ly Dong Thi Ha, Huong Nguyen Mai (2020), "RP-HPLC and UV Spectrophotometric Analysis of Paracetamol, Ibuprofen and Caffeine in Solid Pharmaceutical Dosage Forms by Derivative,Fourier, and Wavelength Transforms: A Comparison Study", Journal of Analytical Methods in Chemistry /Volume 2020,Issue 1.

[3] Hazel Palmer, Garry Graham, Kenneth Williams, and Richard Day, (2010), "A Risk-Benefit Assessment of Paracetamol (Acetaminophen) Combined with Caffeine", Pain medicine 2010; 11:951-965, Wiley Periodicals, Inc.

[4] Anurudhha R. Chabukswar, Vishakha G Thakur, Deepali L.Dharam,Mansi H Shah, Bhanudas S Kuchekar, Shailesh N. (2012), "Development and Validation of HPTLC Method for Simultaneous Estimation of Paracetamol, Ibuprofen and Caffeine in Bulk and Pharmaceutical Dosage Form", Research Journal of Pharmacy and Technology (RJPT) Volume - 5, Issue - 9, Year - 2012

[5] Zhanag WY(2001), "A benefit-risk assessment of caffeine as an analgesic adjuvant.", Europe PMC,1 Jan 2001, 24(15):1127-1142,

[6] Rabia Bushra and Nausheen Aslam, "An Overview of Clinical Pharmacology of Ibuprofen ", Oman Medical Journal 2010, Volume 25, Issue 3, July 2010

[7] Binh Thuc Tran, Tuyen Ngoc Tran, Ai My Thi Tran, Giang Chau Dang Nguyen and Quynh Trang Thi Nguyen (2022), "Simultaneous Determination of Paracetamol, Ibuprofen, andCaffeine in Tablets by Molecular Absorption

SpectroscopyCombined with Classical Least Square Method", Molecules 2022,27,2657.

[8] Dr. Dolly D. Dattani, Dr.Jasuma J Rai (2016), "Comparision of the Efficacy of Ibuprofen to Ibuprofen,Paracetamol and Caffeine Combination in the Control of Postoperative Pain After Periodontal Surgery- ASplit Mouth Cross-Over Study", IJSR - International Journal of Scientific Research, Volume.5, Issue.1,2277-8179.

[9] Gurunath V Pandit, Shailendra V.Dalvi, Gunesh G.Gundi,Shivani G,Gaonkar (2023),, "Simultaneous estimation of Paracetamol, Caffeine, Phenylephrine Hydrochloride and Chlorpheniramine Maleate in pharmaceutical dosage form using High Performance Liquid Chromatography", Journal of Emerging Technologies and Innovative Research (JETIR), June 2023, Volume 10, Issue 6.

[10] ICH Q2 (R1) Guidelines: Validation of Analytical Procedures.

[11] Mahesh Attimarad (2011), "Simultaneous determination of paracetamol and lornoxicam by RP-HPLC in bulk and tablet formulation", Pharmaceutical Methods, January-March, Vol 2, Issue 1.

[12] P.R.Solanki, S.Prachi and S.D.Boob, "RP - HPLC Method for Estimation of Paracetamol from Pharmaceutical Formulation Febrinil", Science Reviews and chemical communications, 2(3), 2012, 232-236.

[13] Pramod Kumar, Amardeep Ankalgi, Pooja Kaushal, Mahendra Singh, (2020), "Method development and validation for Multicomponent analysis of Rosuvastatin Calcium and Losartan Potassium in bulk drug by RP-HPLC", Journal of Drug delivery and Therapeutics (JDDT) 2020;10(5-s);76-84.

[14] Priya Sadapha, Kavita Dhamak, International Journal of pharmaceutical Sciences Review and Research, (2022), "Review Article on High-Performance Liquid Chromatography (HPLC) Method Development and Validation", International Journal of Pharmaceutical Sciences Review and Research, (Int. J. Pharm. Sci. Rev. Res), 74(2), May-June, Article .03, Pages.23-29.

[15] Kayoed Julius, Adebayo (2015), "Effective HPLC method development", Journal of Health, Medicine and Nursing,www.iiste.org, ISSN 2422-8419 (Online) Vol. 12, 2015.

[16] Michael W. Dong, Kim Huynh-Ba, Anissa W. Wong (2020), "Validation of Stability-Indicating HPLC Methods for Pharmaceuticals: Overview, Methodologies, and Case Studies", LCGC North America, LCGC-11-01-2020, Volume 38, Issue 11, Pages: 606–618.

[17] Ms. Nilam Bavdhane, Dr. Vijaya Barge (2024), "A Review on Analytical Method Validation", International Journal of scientific Research in Science and Technology, www.ijsrst.com,Volume 11, Issue 1, 432-444.