ejpmr, 2021,8(12), 601-606

SJIF Impact Factor 6.222

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

GREEN CHROMATOGRAPHIC METHOD DEVELOPMENT AND VALIDATION FOR THE SIMULTANEOUS ESTIMATION OF PARACETAMOL & LORNOXICAM USING HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC) IN BULK

Shivangi[1]*, Jain Neetesh Kumar[1] and Soni Priyanka[2]

[1]Faculty of Pharmacy, Oriental University Indore-India.
[2]Chameli Devi Institute of Pharmacy, Indore-India.

***Corresponding Author: Shivangi**

Oriental University, Indore.

Article Received on 21/10/2021 **Article Revised on 11/11/2021** **Article Accepted on 30/11/2021**

ABSTRACT

A simple Green (eco-friendly), simple, selective, rapid, precise, reverse phase HPLC analytical method is proposed and validated for the simultaneous estimation of Lornoxicam and Paracetamol using environment friendly solvents. The result observed showed that ethanol: phosphate buffer at pH 3.0 adjusted with orthophosphoric acid in 35:65 ratios, at a flow rate of 1.0ml/min was identified as the best chromatographic method for simultaneous estimation of these drugs in line to green analytical separations. The method was developed on Thermostat hypersil C18 column (5 µm, 250mm x 4.60mm) as the stationary phase. The detection wavelength taken was 262.5nm. The method was validated in accordance with the ICH guidelines and validated for linearity, selectivity, LOC, LOQ and robustness. The result showed that the developed method is precise, accurate and can be applied for determination of the same drugs, for routine analysis with affecting the environment.

KEYWORDS: Green chromatography, Lornoxicam, Paracetamol, Simultaneous estimation.

INTRODUCTION

Analytical investigation of bulk drug materials, intermediates, drug ingredients, drug formulations, impurities and degradation products, as well as biological samples containing drugs and their metabolites, is critical in the field of pharmaceutical development.[1]

Green chemistry is concerned with analytical chemists' position in making laboratory activities more environmentally friendly, and it has piqued chemists' interest. Green Analytical Chemistry (GAC) is an important concept that is steadily gaining popularity as a result of increased environmental awareness, as its implementation helps to reduce the negative impact analytical chemistry methodologies may have on the environment.[2] In the research process, miniaturization and automation of analytical methods, reduction (and even disposition) of organic solvents and other harmful reagents, minimization of energy consumption, reduction of wastes and the re-use of solvents and materials are the key trends of green analytical chemistry. In this context, efforts have shifted significantly in recent years to the advancement of green analytical sample preparation and green analytical separation and their measuring techniques.[3] Chromatography is often used in medicinal chemistry to analyze and purify a wide variety of organic molecules. However, the majority of medicinal chemistry waste is produced by chromatographic solvents. Users are also impacted economically with the use of solvents such as DCM which represent great toxicity to humans and severe environmental effects too. Solvents shall be considered as a preliminary component during the analysis and in pharmaceutical separations. Globally, millions of tons of solvents are consumed annually, and the same amounts are consumed year after year.[4] Per day, pharmaceutical companies use approximately 1-1.5 liters of solvents for routine research, which also produces approximately 1-1.5 liters of fluids. Liquid chromatography is a process that is primarily concerned with solvent selection during studies. Solvent collection must be precise in order to conduct HPLC analysis successfully. The most commonly used solvents in HPLC separation are acetonitrile, methanol, water, and THF. Numerous solvents are often harmful to the environment when used in testing. According to the EHS Guidelines, solvents such as methanol, acetonitrile, and THF have a negative long-term effect on the atmosphere.[5] Numerous solvents are carcinogenic, and numerous solvents are hazardous to the environment. Beyond acute or chronic toxicity, the risks associated with solvent use include carcinogenicity, mutagenicity, as well as atmospheric disruption, such as ozone depletion or global climate change.[6]

Alternative solvents must be used to mitigate risk to analysts and the environment in compliance with EHS, GSk, Sanofi, and Pfizer data and directives for analytical purposes. Similarly, chromatographic separations may be performed in the same manner so as to suits the principles of green chromatography.[7] Chemical solvents such as ethanol, isopropanol, n-propanol, acetone, ethyl acetate and propylene carbonate are commonly known by different organizations such as Pfizer or EHS, as the alternatives to be explore during the analytical separations and these solvents are considered as greener solvents as compared to ACN, MeOH etc.

The cost involved in the use of solvents is very important factor. For example as with the case of DCM the users forced to pay an ever-increasing price for the high purity DCM, they are also required to pay high disposal fees for such solvents.[8] and thus this represents extra burden to pharmaceutical industries. So alternative solvents use must be encouraged. Likewise, the use of large quantities of bio solvents characterized by high volatility and toxicity is usually essential for conventional extraction methods and separation techniques. Thus, it is especially important that conventional organic solvents are substituted for less toxic alternatives when new sustainable testing methods are developed. All organic substances that are liquid at ordinary temperatures and are lipophilic are somewhat narcotic. Inhalation of vapors should always be avoided as much as possible. Most common solvents are inflammable to varying degrees. Those with low boiling points or low flash points require special precautions.[9] Transfer of a solvent with low electrical conductivity from a large shipping container to a smaller, ready-use container can be associated with an accumulation of static charge, with the chance that a spark may occur.[10] and thus solvent selection should be done in very precise manner for any analytical operation.

Regarding analytical division, it is well known, as moving stages in liquid chromatography, that huge amounts of harmful organic solvents (mainly acetonitrile) are essential. However, few alternatives have been found to acetonitrile, such as ethanol, 2-propanol or propylene carbonate. These alternative solvents exhibit high viscosity or low water miscibility, leading to pressures in the LC system. The use of LC mobile additives to change the essence of the analytes' interactions with the mobile phase and/or the stationary phase would help improve the separation and the lowering of the solvent intake by an alternative to improve the sustainability of LC methods. Additional methods include using high-performance LC or nano-LC to lower solvents even further, along with a remarkable reduction in spacing times, and designing new stationary phases in chromatography to provide acceptable performance with low organic solvent demands.[11],[12],[4]

Development and evaluation for research methodologies require the optimization of certain important analytical parameters (e.g., accuracy, sensitivity, reproducibility, simplicity, cost effectiveness, flexibility and speed) as per the guidelines provided by ICH (ICH guidelines Q2/R1). However other aspects relating to operator protection and environmental effects of analytical approaches are not usually taken into account. Due to the side-effects of analytical methodologies, which were designed to analyze various samples, including the biological and pharmaceutical samples, which produce a large amount of chemical waste and have a large environmental and human impact. Chemicals used in the study, in certain circumstances become more harmful than the substance to be analyzed.[13] Thus here in present work we want to apply the principles of green analytical method development and to design and develop a precise, simple, cost effective green analytical method for the simultaneous estimation of paracetamol & lornoxicam drugs in bulk as well as combined dosage form.

Paracetamol, also known as Acetaminophen or Tylenol, is an analgesic-antipyretic N-acetyl-para-aminophenol and para-acetyl-aminophenol derivative. It works well for mild-to-moderate pain, including headaches, neuralgia, and musculoskeletal pain. Paracetamol is a widely prescribed pain reliever that can also be used to lower body temperature. Since it is safe for most people to take and has few side effects, paracetamol is often used as one of the first remedies for pain and an over the counter medication.[14][15] Lornoxicam is a nonsteroidal anti-inflammatory drug with analgesic and antipyretic properties that belongs to the class of oxicams. It acts by nonselective inhibition of cyclo-oxygenase-1 and -2. It is prescribed for osteoarthritis, rheumatoid arthritis, acute lumbar-sciatica conditions, and for postoperative pain management.[16], [17] The drug combination Paracetamol and lornoxicam is given as fixed dose combination for treating arthritis, and inflammatory disorders to relieve pain. From the literature survey it was found that a number of spectrophotometric and chromatographic methods have been reported for the combination assessment but not a single report was there which involves the use of green solvents like ethanol.[18],[19],[20],[21] So here an attempt was made to develop a simple environment friendly green chromatographic method for the simultaneous estimation of the two drugs in combination dosage form.

EXPERIMENTAL

Instrument utilized

Liquid chromatographic system utilized is the LC-10ATvp Schimadzu with manual injector, with pump Solvent Delivery model LC-10ATvp for constant flow and constant pressure delivery and SPD-M10 Avp-Schimadzu, UV/Vis Diode Array Detector connected to software LC solution for controlling the instrumentation was used.

Reagents and chemicals

Samples of Paracetamol and Lornoxicam were obtained as gift sample from Glenmark Pharmaceuticals Ltd, Mumbai, India. Ethanol, Methanol, Sodium hydroxide, Potassium dihydrogen orthophosphate, triethylamine and orthophosphoric acid were supplied by Merck Chemicals, Mumbai, India. The solvents and the reagents used here are all HPLC grade or analytical grade. Triple Distilled water was obtained in house and used. The fixed dose drug combination used for the assay was procured from local market Lorsaid P which was manufactured by Abbott India Ltd and it contains Lornoxicam (8mg) & Paracetamol (325mg).

Chromatographic condition

Taking into consideration the system suitability parameters like RT, Tailing Factor, No. of theoretical plates and HETP, the mobile phase found to be most suitable for analysis was ethanol: phosphate buffer pH 3.0 (pH adjusted with ortho-phosphoric acid) in the ratio of 35:65 respectively. The mobile phase was filtered through 0.45um filter paper to remove the particulate matter if any and then degassed by sonication. Flow rate employed for the analysis was 1.0ml/min. the column used as stationary phase is Thermostat hypersil C18 column (5 µm, 250mm x 4.60mm). After observing the UV spectrum of both the drugs and their overlain spectra as well considering the chromatographic parameters, 262.5nm was considered as the λmax for all the determinations for UV/Vis Diode Array Detector.

Standard preparation

1). Preparation of Phosphate buffer (pH 3.0): Dissolved 1.36gms of potassium dihydrogen orthophosphate and 2 ml of triethylamine in 800ml of water and adjusted the pH to 3.0 with orthophosphoric acid and added sufficient water to produce 1000ml with distilled water.

2). Preparation of standard stock solution of PCM & LOX (1000 µg/ml**):** 100 mg of paracetamol and lornoxicam were accurately weighed and transferred to 100 ml volumetric flask separately and dissolved in ethanol: phosphate buffer and sonicated for 5 min.

3). Preparation of working standard: From the stock solution prepared above different aliquots were taken and diluted with solvent system to obtain different concentration viz 5, 10, 20, 30, 40, 50 µg/ml for paracetamol and 4, 8, 16, 24, 32, & 40 µg/ml for Lornoxicam.

Sample preparation

For the assay of marketed drug dosage form Twenty tablets of Lorsaid P were taken, weighed individually and grounded to make fine powder. An accurately weighed powder sample equivalent to 8 mg of LOX and 325 mg PCM were transferred to 100 ml of volumetric flask and mixed with little quantity (approx. 40ml) of mixture of diluent. The above solution was degassed & sonicated for about 10 min to solubilize the drugs completely in the diluent and the volume was made up to the mark and filtered through Whatman filter paper No. 42, finally different concentrations of tablet sample were prepared by serial dilution technique.

RESULTS AND DISCUSSION

Chromatography

After several trials with ethanol, ethylacetate, isopropanol, water and buffer solution in different ratios and at different pH, the mobile phase was selected for the analysis. The final mobile phase consists of ethanol: phosphate buffer (35:65 at pH 3.0). Flow rates employed during the analysis was between 0.5 and 1.5ml/min but finally flow rate of 1.0 ml/min has been selected to get the optimized signal to noise ratio and for precise system suitability. Using a RP C18 column, the retention times for PCM and LOX were observed to be 3.63 and 5.17 min, respectively. Total time of analysis was less than 10 min. From the overlain spectra observed during the analysis 262.5nm wavelength was selected for complete analysis.

System suitability

Separation variables were fixed and mobile phase was allowed to run through the column at flow rate of 1.0 ml/min for complete saturation of column, six replicates of calibration standards of LOX and PCM were injected separately. Peak report, HETP, Peak Tailing & column performance report were recorded for all chromatograms. The number of theoretical plates observed for PCM & LOX were 7453 & 3574 respectively.

Table 1: System suitability criteria.

System suitability parameter	PCM	LOX
Retention time ± %RSD	3.62 ± 0.26	5.16 ± 0.42
Theoretical plates ± %RSD	7453 ± 0.42	3574 ± 0.12
Tailing Factor ± %RSD	1.43 ± 1.39	1.41 ± 0.82
AUC ± %RSD	1498481 ± 0.007	109842 ± 0.076
Linearity in µg/ml	5-50	4-40
LOD(µg/ml)	0.8	1.0
LOQ (µg/ml)	1.0	1.5

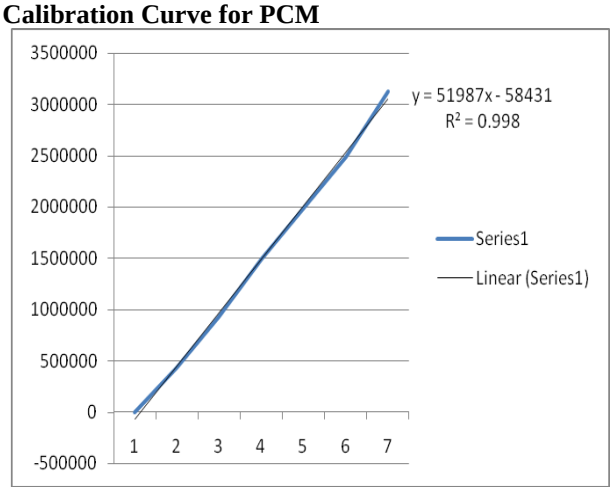
Linearity

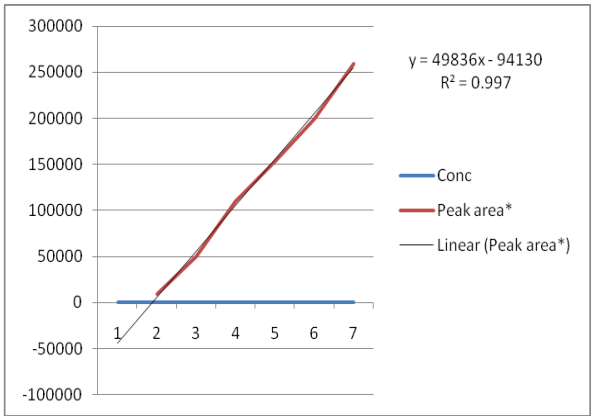
PCM and LOX showed a linearity of response between 5-50 and 4-40 µg/ml, respectively. The linearity was represented by a linear regression equation. The results of statistical analysis were shown in Table 2.

Table 2: Statistical analysis: Linearity.

Parameter	PCM	LOX
Linearity Range, µg/ml	5-50	4-40
Slope	51987	49836
Intercept	-58431	-94130
Regression (R^2)	0.998	0.997

Calibration Curve for PCM





Calibration Curve for LOX

Y (PCM) = 51987conc. - 58431 (r2=0.998)

Y (LOX) = 49836 conc. - 94130 (r2=0.997)

Where, Y is area under curve and r2 is correlation coefficient

Accuracy

Accuracy of the method was calculated by recovery studies at three levels (n=3, 80%, 100%, 120%) by standard addition method Table 3. The mean percentage recoveries obtained for LOX and PCM were 98.33-102 % and 98.16-102.5%, respectively.

Table 3: % Recovery study of PCM & LOX.

S. No.	Amount of drugs in preanalysed samples µg/ml		Amount of std drug added in µg/ml		Total amount of the drug recovered in µg/ml		% Recovery	
	PCM	LOX	PCM	LOX	PCM	LOX	PCM	LOX
1	5	5	4	4	8.99	9.01	99.75	102.5
2	5	5	5	5	10.1	10.01	102.0	100.2
3	5	5	6	6	11.1	10.89	98.33	98.16

Precision Study

a). Repeatability: Six replicates were analyzed in same day for repeatability and results were found within acceptable limits (relative standard deviation, RSD ‹ 2) as shown in Table 4.

b) Intermediate precision: The five successive injection of given drug concentration were injected on the same day to determine intra-day precision and Inter-day precision was studied by repeating the studies on different days (4 days) and results were found within acceptable limits (RSD ‹ 2) as shown in Table 4.

c) Robustness: Robustness study is carried out by making slight changes in pH (2.9 and 3.1) of mobile phase and alteration in the flow rate from 1.0ml/min to 0.9 ml/min and 1.1 ml/min. The percentage assay when the pH was changed was found to be 96.68% - 97.64 % for Paracetamol & 98.65 % - 100.15% for Lornoxicam and On varying the flow rate to 0.9 ml/min and 1.1 ml/min, the % assay was found to be in the range of 103.62 % - 98.66 % for Paracetamol & 104.03% - 97.53% for Lornoxicam respectively.

Table 4: Precision Study of PCM & LOX.

Validation parameter	Percentage Mean*± S. D.		Percentage RSD*	
	PCM	LOX	PCM	LOX
Repeatability	30.48± 0.4292	24.35 ± 0.155	0.014	0.006
Intermediate precision Day to day	30.44± 0.20	24.65± 0.31	0.68	1.2
Intra-day	30.52 ± 0.47	24.24± 0.17	1.5	0.72

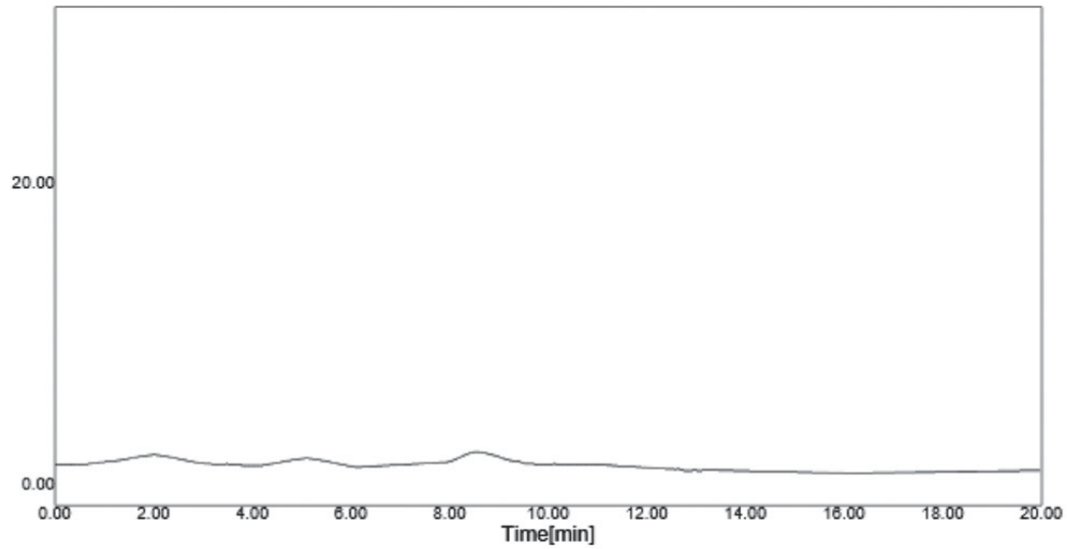
- *Average of three readings

Tablet analysis

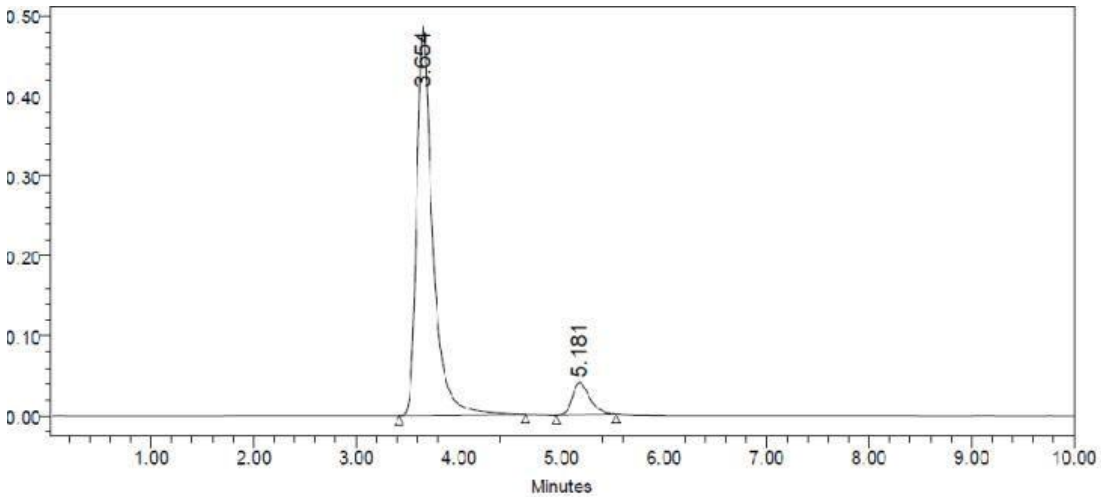
Content of PCM and LOX found in the tablets by the proposed method are shown in Table 5. The percentage assay was found to be 99.81 & 100.03 for Lornoxicam & Paracetamol respectively.

Table 5: Result of analysis of Marketed formulation.

Formulation	Label Claim (mg)		Amount Found (µg/ml)		% Assay	
	LOX	PCM	LOX	PCM	LOX	PCM
Lorsaid P	8	325	7.98	325.09	99.81	100.03



Chromatogram of the Blank



Chromatogram of the tablet formulation

CONCLUSIONS

In the proposed work a green simple RP-HPLC method was developed and validated for simultaneous estimation of Paracetamol and Lornoxicam in tablet dosage form. The column used here was C18 column and after a several trials, considering different combination of mobile phases, in different ratios and at different pH, the ethanol: phosphate buffer at pH 3.0 (adjusted with orthophosphoric acid) in ratio of 35:65 was selected as mobile phase, which is said to be the greener mobile phase system. The system suitability parameters indicated that the number of theoretical plates were well above 2000 as well as tailing factor is less than 2, which shows the suitability of the analytical method. The developed method shows a linearity range of 5-50 µg/mL for pCM and 4-40 µg/mL for Lornoxicam with regression values of 0.998 & 0.997 for PCM & LOX respectively, showing the response as linear. The percentage recovery study indicates the accuracy of the method as well as the low % RSD values of Precision are within the acceptable range (% RSD < 2), indicates the high degree of precision of the method. The assay in the tablet formulation reveals that there is no interference or overlapping of any peak indicating the specificity of the method for simultaneous estimation of the drugs. The total run time of the method is less than two minutes for elution of both the drugs. Slight variation in the flow rate and pH range doesn't cause any significant change of analytical results indicating the robustness of the method. So, here, using the Green solvents as mobile phase (Ethanol: Phosphate buffer), a fast, accurate, precise and robust method was developed and the same can be employed for routine analysis of these drugs in industries as well as academic institutions.

REFERENCES

1. Siddiqui MR, AlOthman ZA, Rahman N. Analytical techniques in pharmaceutical analysis: A review. Arabian Journal of Chemistry, 2017; 2017/02/01/; 10: S1409-S21.
2. Gałuszka A, Migaszewski Z, Namieśnik J. The 12 principles of green analytical chemistry and the SIGNIFICANCE mnemonic of green analytical practices. TrAC Trends in Analytical Chemistry, 2013; 2013/10/01/; 50: 78-84.
3. Buncel E, Stairs RA. Role of the Solvent in Chemical Reactions: Second Edition, 2015; 1-227.
4. Vian M, Breil C, Vernes L, Chaabani E, Chemat F. Green solvents for sample preparation in analytical chemistry. Current Opinion in Green and Sustainable Chemistry, 2017; 2017/06/01/; 5: 44-8.
5. Hassan B. HPLC Uses and Importance in the Pharmaceutical Analysis and Industrial Field. Pharmaceutica Analytica Acta, 2012; 01/01; 03.
6. Boligon A, Linde M, editors. Importance of HPLC in Analysis of Plants Extracts, 2014.
7. Karger BL. HPLC: Early and Recent Perspectives. Journal of Chemical Education, 1997; 1997/01/01; 74(1): 45.
8. Mohammad A, Inamuddin, Siddiq A, Highly Cited Researcher M, El-desoky G. Green Solvents I., 2012; 331-61.
9. Yabré M, Ferey L, Somé IT, Gaudin K. Greening Reversed-Phase Liquid Chromatography Methods Using Alternative Solvents for Pharmaceutical Analysis. Molecules, 2018; 23(5): 1065. PubMed PMID: 29724076. eng.
10. Huang Y, Yao S, Song H. Application of Ionic Liquids in Liquid Chromatography and Electrodriven Separation. Journal of chromatographic science, 2013; 51(7): 739-52.
11. Pacheco-Fernández I, Pino V. Green solvents in analytical chemistry. Current Opinion in Green and Sustainable Chemistry, 2019; 2019/08/01/; 18: 42-50.
12. Tobiszewski M, Namieśnik J. Greener organic solvents in analytical chemistry. Current Opinion in Green and Sustainable Chemistry, 2017; 2017/06/01/; 5: 1-4.
13. Armenta S, Garrigues S, de la Guardia M. Green Analytical Chemistry. TrAC Trends in Analytical Chemistry. 2008, 2008/06/01/; 27(6): 497-511.
14. https://pubchem.ncbi.nlm.nih.gov/compound/Acetaminophen.
15. https://go.drugbank.com/drugs/DB09288.
16. https://pubchem.ncbi.nlm.nih.gov/compound/Lornoxicam, https://go.drugbank.com/drugs/DB06725.
17. http://www.chemspider.com/Chemical-Structure.10442760.html.
18. Babar SJ, Mane VB, Bhise SB. Simultaneous estimation of lornoxicam and paracetamolin tablets by reverse phase HPLC method. Der Pharma Chemica, 2013; 01/01; 5: 103-8.
19. Attimarad M. Simultaneous determination of paracetamol and lornoxicam by RP-HPLC in bulk and tablet formulation. Pharm Methods, 2011; 2(1): 61-6. PubMed PMID: 23781432. eng.
20. Jain P, Patel M, Chaudhari A, Surana S. RP-HPLC assay method development for Paracetamol and Lornoxicam in combination and characterization of oxidative degradation products of Lornoxicam. Chemical Industry and Chemical Engineering Quarterly, 2013; 10/01; 19: 471-84.
21. Kumar Talluri MVN, Bairwa MK, Theja Dugga HH, Srinivas R. DEVELOPMENT AND VALIDATION OF RP-HPLC AND ULTRAVIOLET SPECTROPHOTOMETRIC METHODS OF ANALYSIS FOR SIMULTANEOUS DETERMINATION OF PARACETAMOL AND LORNOXICAM IN PHARMACEUTICAL DOSAGE FORMS. Journal of Liquid Chromatography & Related Technologies, 2012; 2012/01/01; 35(1): 129-40.