Research Article

# Development and Method Validation of Lumateperone by UV Spectroscopy and Forced Degradation Study

**Dr.Waghmare S.S*, Kawadkar N.G, J. Jagtap P.B, Devne S.R, Bhagdewani N.S, Kherade D.S, Dr.Ingale P.L**
SGMSPM's Dnyanvilas College of Pharmacy, Dudulgaon, PCMC, Pune, MS, India.
***Corresponding author's E-mail:** smita.waghmare31@gmail.com

**Received:** 12-10-2024; **Revised:** 28-12-2024; **Accepted:** 09-01-2025; **Published online:** 20-01-2025.

**ABSTRACT**

Simple, precise, accurate and economical UV Spectrophotometric method have been developed for Lumateperone an Atypical Antipsychotic Drug approved for treatment of bipolar disorder and schizophrenia. Less expensive solvent like methanol is used for method validation study. Method validation study was done using UV-VIS spectrophotometer (make-Shimadzu) at 243.00 nm wavelength. Drug Lumateperone obeyed Beer- Lambert's law. Drug concentration ranges from 5µg/ ml- 45µg/ ml were selected for study. Coefficient of correlation was found to be 0.9999, precision of method with RSD% of 0.145797, accuracy of method with 99.525 recovery, LOD is 0.315 and LOQ is 0.964. Lumateperone was subjected to forced degradation under different condition as per ICH guidelines. The sample generated was used for degradation studies using developed method.

**Keywords:** Lumateperone, Development and method validation, UV spectroscopy.

## INTRODUCTION

Lumateperone is an atypical Antipsychotic Drug of butyrophenone class. It is approved for the treatment of bipolar depression and schizophrenia. It is a white to off white crystalline powder, soluble in methanol, DMSO, Dimethyl Formamide (DMF). A chemical structure of Lumateperone as shown in figure 1.

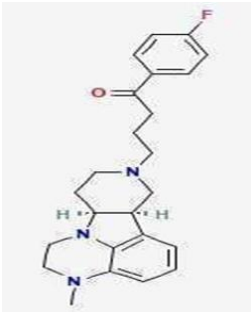


**Figure 1:** Lumateperone

The aim of this work is to develop and validate stability indicating method by using UV-VIS Spectrophotometry in bulk Lumateperone and also perform degradation studies on drug as per ICH guidelines. Lumateperone approved by the U.S. Food and Drug Administration (FDA) for the treatment of schizophrenia in adults Lumateperone has a unique mechanism of action, which involves simultaneous modulation of serotonin, dopamine, and glutamate neurotransmission. [1]Method validation is a crucial process in ensuring the accuracy, precision, and reliability of analytical methods used to quantify drugs like lumateperone in biological samples or pharmaceutical formulations. For lumateperone, method validation typically involves parameters such as specificity, linearity, accuracy, precision, limit of detection (LOD), limit of quantitation (LOQ), and stability.[2] Acid degradation studies are part of forced degradation studies used to determine the stability of pharmaceutical compounds like lumateperone under acidic conditions. These studies help in understanding the degradation pathways and developing stability-indicating methods.[3]

## MATERIALS AND METHODS

Lumateperone sample was obtained from Dr. Reddy Laboratory. The instrument used was UV-Vis double beam Shimadzu Corporation, high speed scanning spectrophotometer. The solvent used was distilled water, NaOH, HCl and $H_2O_2$. These chemicals were purchased from Oxford Lab Fine Chem LLP

**UV method development:-**

*Preparation of standard solution Lumateperone:*

Standard stock solution of Lumateperone (100 µg/ml) was prepared by accurately weighing about 10 mg of drug dissolved in 100ml of methanol and makeup volume in 100 ml of volumetric flask up to the mark with the same solvent.

**Dilutions:**

**Table 1:** Concentration Table of Lumateperone

| Concentration | Stock Solution | Final Volume |
|---|---|---|
| 5µg/ ml | 0.5ml | 10ml |
| 10µg/ ml | 1ml | 10ml |
| 15µg/ ml | 1.5ml | 10ml |
| 20µg/ ml | 2ml | 10ml |
| 25µg/ ml | 2.5ml | 10ml |
| 30µg/ ml | 3ml | 10ml |
| 35µg/ ml | 3.5ml | 10ml |
| 40µg/ ml | 4ml | 10ml |
| 45µg/ ml | 4.5ml | 10ml |

**Method of validation:**

**1) Linearity:**

Various aliquots were prepared from the stock solutions (100 µg/ml) ranging from 5-45 µg/ml for both Lumateperone. The samples were scanned in UV Spectrophotometer against Methanol as blank. The calibration curves of Absorbance versus Concentration were plotted and correlation coefficient and Regression line equations for Lumateperone was calculated and given in (Fig 2, Graph 1 and Table 2).

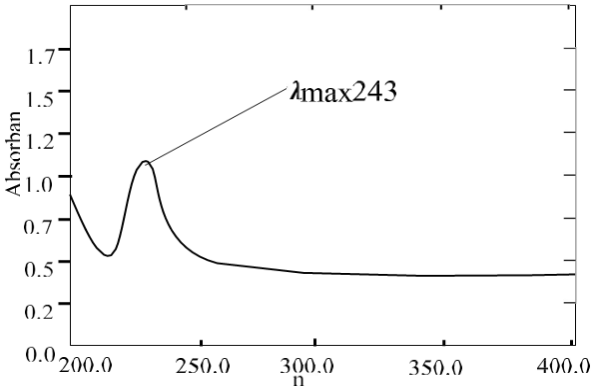
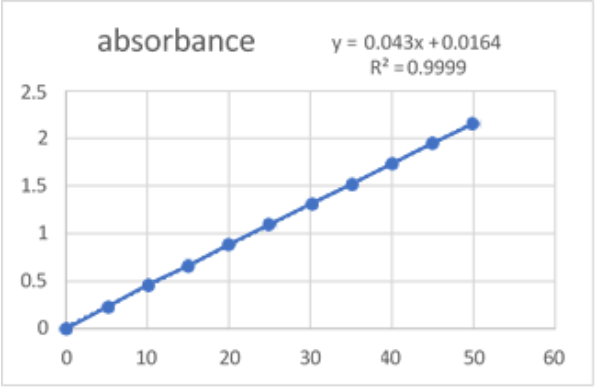



**Figure 2:** UV Spectrum of Lumateperone at 243nm




**Graph 1:** Linearity graph of Lumateperone

**Table 2:** Optical and Regression Characteristics of Lumateperone

| Parameters | Lumateperone (243nm) |
|---|---|
| Beer's law Limit (µg/ml) | 5-45 µg/ml |
| Slope (a) | 0.043 |
| Intercept (b) | 0.0164 |
| Correlation Coefficient ($R^2$) | 0.9999 |
| Regression Equation | y = 0.043x + 0.0164 |

**2) Precision:**

**a. Repeatability:** To check the degree of repeatability, standard solution containing Lumateperone were taken six times separately of the same concentration 20µg/ml and was analyzed. The standard deviation (S.D.) and Percent Relative Standard Deviation (% R.S.D.) was calculated and given in Table 3.

**b. Intra-day precision:** The intra-day precision checked by analyzing the sample solutions of same concentration for three times on the same day with in short interval of time and S.D. and % R.S.D. was calculated and given in Table 4.

**c. Inter-day precision:**

The inter-day precision checked by analyzing the sample solutions of same concentration for three times on the three different days with in short interval of time and S.D. and % R.S.D. was calculated given in Table 5.

**3) Robustness**:

Robustness of the proposed method is determined by making the deliberate variation in method parameter such as change in wavelength and S.D. and % R.S.D. was calculated given in Table 6.

**4) Ruggedness**:

Ruggedness of the method was determined by carrying out the analysis by three different analysts. The respective absorbance was noted and S.D. and % R.S.D. was calculated given in Table 7.

**Table 3:** Repeatability data of Lumateperone

| Sr. No. | Conc. Taken (µg/ml) | Absorbance | Conc. Found (µg/ml) | %Purity | S.D. | % R.S.D. |
|---|---|---|---|---|---|---|
| 1 | 20 | 0.8815 | 20.1186 | 100.593 | 0.146451 | 0.145797 |
| 2 | 20 | 0.8789 | 20.05814 | | | |
| 3 | 20 | 0.87956 | 20.07349 | | | |
| 4 | 20 | 0.879632 | 20.07516 | | | |
| 5 | 20 | 0.8817 | 20.12326 | | | |
| 6 | 20 | 0.880258 | 20.08973 | | | |

**Table 4:** Intra-day precision data of Lumateperone

| Interval | Conc.( g/ml) | Absorbance | Conc. Found (µg/ml) | Purity | S.D. | R.S.D. |
|---|---|---|---|---|---|---|
| Morning | 20 | 0.880158 | 20.0874 | 00.3798 | 0.108755 | 0.107799 |
| Afternoon | 20 | 0.881024 | 20.10753 | 00.6541 | | |
| Evening | 20 | 1.02456 | 15.06976 | 00.3565 | | |

**Table 5:** Inter-day precision data of Lumateperone

| Interval | Conc. Taken (µg/ml) | Absorbance | Conc. Found (µg/ml) | % Purity | S.D. | % R.S.D. |
|---|---|---|---|---|---|---|
| Day 1 | 20 | 0.88478 | 20.19488 | 100.3565 | 0.161464 | 0.163148 |
| Day 2 | 20 | 0.87945 | 20.07093 | | | |
| Day 3 | 20 | 0.87858 | 20.0507 | | | |

**Table 6:** Robustness data of Lumateperone

| λmax (nm) | Conc.Taken (g/ml) | abs | Conc. Found (µg/ml) | % Purity | S.D. | R.S.D. |
|---|---|---|---|---|---|---|
| 240 | 20 | 0.8831 | 20.156 | 100% | 0.1955 | 0.194 |
| 245 | 20 | 0.8741 | 19.94 | 99% | 0.5614 | 0.559 |

**Table 7:** Ruggedness data of Lumateperone

| Analyst | Conc. taken (g/ml) | Absorbance Found (µg/ml) | Conc. Found (µg/ml) | Purity | S.D. | R.S.D. |
|---|---|---|---|---|---|---|
| **Analyst I** | 20 | 0.8599 | 9.61628 | 8.0814 | 1829 | 0.1848 |
| **Analyst II** | 20 | 0.8669 | 9.77907 | 8.89535 | 1132 | 0.1143 |
| **Analyst III** | 20 | 0.8756 | 9.9814 | 9.90698 | 1337 | 0.1351 |

**Table 8:** Recovery study data of Lumateperone

| Sr.No. | Concentration Level % | Total amount (µg/ml)) | Amt of drug found (µg/ml) | Amt of drug recovered (µg/ml) | S.D. | R.S.D. | % recovery |
|---|---|---|---|---|---|---|---|
| 1 | 0% | 20 | 15 | 34.856 | 0.1889 | 0.1845 | 0.056 |
| 2 | | 20 | 15 | 35.7561 | | | |
| 3 | | 20 | 15 | 35.412 | | | |
| 4 | 0% | 20 | 20 | 40.769 | 0.1895 | 0.1896 | 0.095 |
| 5 | | 20 | 20 | 39.489 | | | |
| 6 | | 20 | 20 | 40.824 | | | |
| 7 | 0% | 20 | 25 | 45.248 | 0.1995 | 0.1994 | 0.098 |
| 8 | | 20 | 25 | 45.175 | | | |
| 9 | | 20 | 25 | 45.724 | | | |

**5) Accuracy**:

The accuracy of the method was determined by calculating recovery of Lumateperone by the standard addition method. Known amounts of standard solutions of Lumateperone were added at 80, 100, and 120% levels and % recovery was calculated given in Table 8.

**6) Limit of detection (LOD):**

The LOD was calculated mathematically by using a formula LOD= 3.3*(SD/Slope) given in Table 9.

**Table 9:** LOD data of Lumateperone

| Drug | Lumateperone |
|---|---|
| **LOD (µg/ml)** | 0.315 |

**7) Limit of quantification (LOQ):**

The LOQ was calculated mathematically by using a formula LOQ= 10*(SD/Slope) given in Table 10.

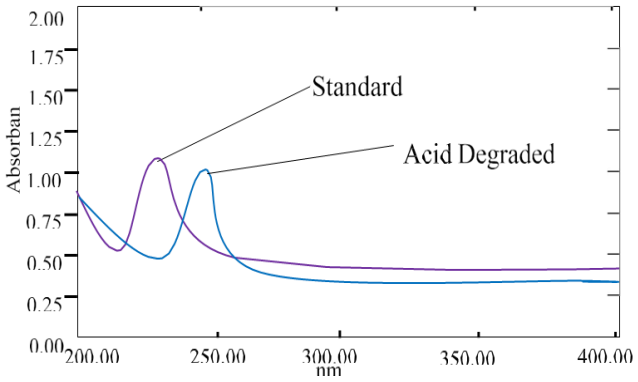
**Table 10:** LOQ data of Lumateperone

| Drug | Lumateperone |
|---|---|
| **LOQ (µg/ml)** | 0.964 |

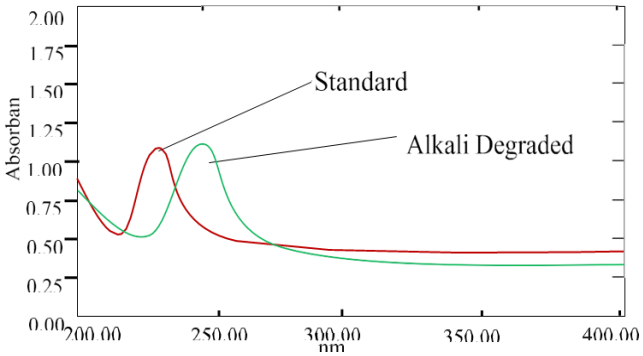
**Stress Degradation Studies:**

1. Acidic Degradation: 5ml of stock solution of Lumateperone, and 5 ml of 2 N HCl were added in 10 ml of volumetric flask and the volumetric flask was kept at 80$^{o}$C for 2 hour reflux and left them for the 30 minutes. Afterwards the absorbance of solution was analyses separately at wavelength max 241nm given in (Graph 2).

2. Alkali Degradation: 5 ml of stock solution of Miconazole and 5 ml of 2 N NaOH was added in 10 ml of volumetric flask and the volumetric flask was kept at 80□c for 2 Hour reflux and left them for the 30 minutes. Afterwards the absorbance of solution was analyses separately at wavelength max 241nm.

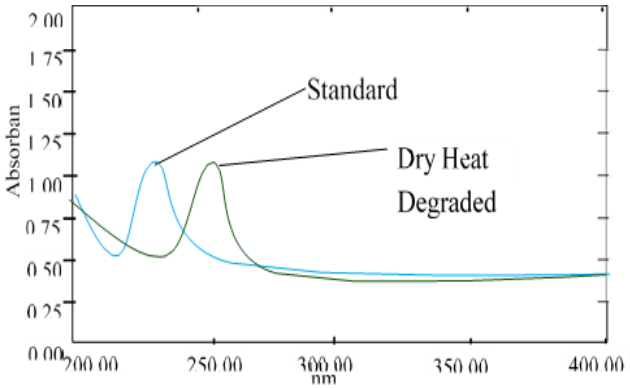



**Graph 2:** Standard vs. Acid Degraded Sample of Lumateperone




**Graph 3:** Standard vs. Alkali Degraded Sample of Lumateperone

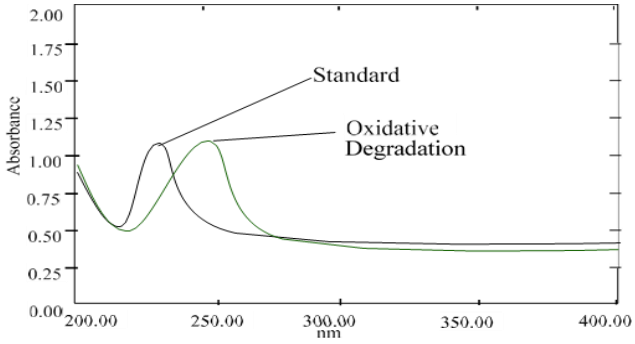
3. Dry Heat Induced Degradation: Lumateperone sample was taken in a petriplate and exposed to a temperature of 50°C for 3 hours in an oven. After 3 hours, 10 mg of the sample was diluted with phosphate buffer up to 10 ml. From this solution, dilution was done to achieve the appropriate concentration (5µg/ml) Graph 4.



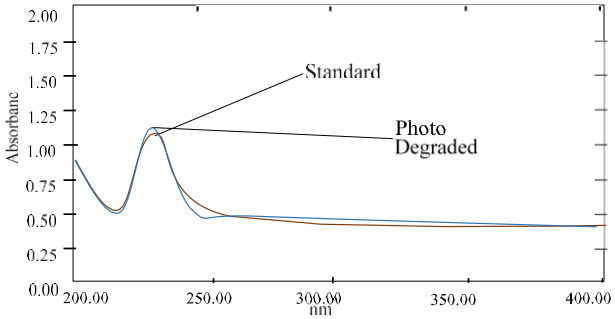

**Graph 4:** Standard vs. Dry Heat Degraded Sample of Lumateperone

4. Oxidative Degradation: Applying 3% of hydrogen peroxide ($H_2O_2$) at room temperature (25°C) and left them for the 3 hour. Afterwards the absorbance of solution were analyses separately at wavelength max 241nm. (Graph 5)

5. Photo-Degradation: Lumateperone sample was taken in a petriplate and exposed to a shorter & longer (230 & 240 respectively) in UV chamber for 3 hrs. 10 mg of the sample was diluted with phosphate buffer up to 10 ml. From this solution, dilution was done to achieve the appropriate concentration (5 µg/ml) (Graph 6).



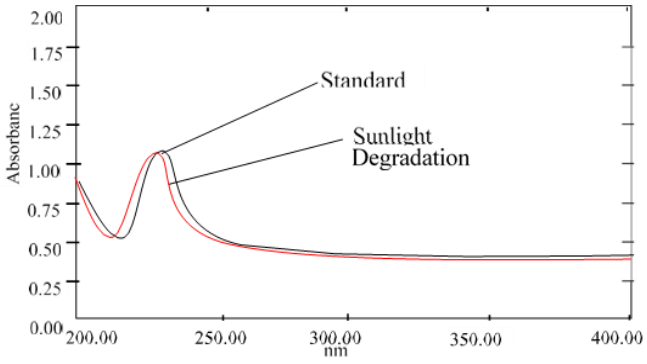

**Graph 5:** Standard vs. Oxidative Degraded Sample of Lumateperone




**Graph 6:** Standard vs Photo Degraded Sample of Lumateperone

Sunlight Degradation: Miconazole sample was taken in a petriplate and exposed to sunlight for 3 hrs. 10 mg of the sample was diluted with phosphate buffer up to 10 ml.

From this solution, dilution was done to achieve the appropriate concentration (5µg/ml) (Graph 7).




**Graph 7:** Standard vs Sunlight Degraded Sample of Lumateperone

## RESULT AND DISCUSSION

The developed method was found to be precise as the %RSD values for intraday and inter-day were found to be less than 2% recoveries of the drug, indicating that the method was accurate. The method was also found to be specific. The LOD & LOQ was found to be in sub microgram level indicating sensitivity of method. The method was found to be robust and rugged as indicated by the %RSD values which

are less than 2%. The stress degradation in acidic, alkali, dry heat (3.45%, 9.74%, 17.84% respectively).

## CONCLUSION

The presented method was validated in terms of reproducibility, sensitivity, accuracy, precision and detection of limits in accordance with internationally accepted guideline, which can be directly easily applied to the analysis of pharmaceutical dosage form of Lumateperone. This method can be used for the routine quality control of the drug in bulk as well as in pharmaceutical formulations. Although no attempt has been made to identify the degradation products of the pharmaceutical dosage form of Lumateperone, the proposed method can still be utilized for stability-indicating analysis. The proposed method can be used as a stability indicating method for assay of Lumateperone in bulk dosage form as well as pharmaceutical formulations and therapeutic drug monitoring of schizophrenic patient.

**Source of Support:** The author(s) received no financial support for the research, authorship, and/or publication of this article

**Conflict of Interest:** The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

**Table 11:** Result of Validation parameters of Lumepterone

| Sr. No. | Parameters | Lumateperone |
|---|---|---|
| 1 | Absorbance Maxima (nm) | 243 |
| 2 | Linearity Range (µg/ml) | 5-45 |
| 3 | Regression equation | y = 0.043x + 0.0164 |
| 4 | Correlation Coefficient ($R^2$) | 0.9999 |
| 5 | Slope (m) | 0.043 |
| 6 | Intercept (c) | 0.0164 |
| 7 | Repeatability % R.S.D. | 0.145797 |
| 8 | Intra-Day Precision % R.S.D | 0.107799 |
| 9 | Inter-Day Precision % R.S.D. | 0.163148 |
| 10 | Recovery (at 80% level) | 100.056 |
| 11 | % Recovery (at 100% level) | 99.525 |
| 12 | % Recovery (at 120% level | 100.098 |
| 13 | Robustness % R.S.D. (at 240nm) | 0.194052 |
| 14 | Robustness %R.S.D. (at 245nm) | 0.559469 |
| 15 | Ruggedness % R.S.D. | 0.1447916 |
| 16 | LOD (µg/ml) | 0.315 |
| 17 | LOQ (µg/ml) | 0.964 |

**Table 12:** Result of Stress Degradation Study

| Stress Condition | Time | Observation | Degradation |
|---|---|---|---|
| Acidic Degradation | RT for 3hr | λmax shifted | 13.45% |
| Alkali Degradation | RT for 3 hr. | λmax shifted | 9.74% |
| Dry Heat Induced Degradation | RT for 3 hr. | λmax shifted | 17.84% |
| Photo-Degradation | For 3 hr. | λmax not shifted | 7.48% |
| Sunlight Degradation | For 3 hr. | λmax not shifted | 0% |

## REFERENCES

1. Kane JM, Vanover KE, Durgam S, Davis R, Satlin A, Rowe W, Mates S, Tamminga C. 185 The Safety and Tolerability of Lumateperone 42 mg for the Treatment of Schizophrenia: A Pooled Analysis of 3 Randomized Placebo-Controlled Trials. CNS spectrums. 2020 Apr;25(2):316-7 DOI: https://doi.org/10.1017/S1092852920001005

2. Gupta, S., Singh, S. K., & Verma, R. Development and validation of a stability-indicating

RP-HPLC method for the determination of lumateperone in bulk and pharmaceutical dosage forms.Journal of Pharmaceutical and Biomedical Analysis, 2021;195:113837 DOI:10.1016/j.jpba.2010.10.002

3. Muzammal M, Firoz A, Ali HM, Farid A, Khan MA, Hakeem KR. Lumateperone Interact with S-Protein of Ebola Virus and TIM-1 of Human Cell Membrane: Insights from Computational Studies. Applied Sciences. 2022 Sep 2;12(17):8820. https://doi.org/10.3390/app12178820

4.Babu KN, Mrinalini TS. Contemporary HPLC-UV reverse phase method development and validation of anti-psychotic drug Lumateperone Tosylate, followed by forced degradation studies. Atomic Spectroscopy-Part C. 2023 Oct 21;44(3):1-1.

5.Dutheil S, Watson LS, Davis RE, Snyder GL. Lumateperone normalizes pathological levels of acute inflammation through important pathways known to be involved in mood regulation. Journal of Neuroscience. 2023 Feb 1;43(5):863-77. https://doi.org/10.1523/JNEUROSCI.0984-22.2022

6. Hamrapurkar P, Patil P, Desai M, Phale M, Pawar S. Stress degradation studies and development of a validated stability-indicating-assay-method for determination of diacerein in presence of degradation products. Pharmaceutical Methods. 2011

Jan 1;2(1):30-5. https://doi.org/10.4103/2229-4708.81088

7. Bangarpoonam Shivaji,. Development and Validation of RP-HPLC Method for the Estimation of Lumateperone Drug in Pharmaceutical Dosage Forms. International Journal of Pharmaceutical Research and Applications. 2023;8:2105-2115

8. Verma G, Mishra M. Development and optimization of U.V-Vis spectroscopy-a review. World J. Pharm. Res. 2018 Apr 19;7(11):1170-80.

9. Adegoke OA. Chemical derivatization methodologies for UV-visible spectrophotometric determination of pharmaceuticals. Int. J. Pharm. Sci. Rev. Res. 2012;14(2):6-24.

10. Blessy MR, Patel RD, Prajapati PN, Agrawal YK. Development of forced degradation and stability indicating studies of drugs—A review. Journal of pharmaceutical analysis. 2014 Jun 1;4(3):159-65. https://doi.org/10.1016/j.jpha.2013.09.003

11. Baertschi SW. Forced degradation and its relation to real time drug product stability. Pharmaceutical Stability Testing to Support Global Markets. 2010:107-16.

12. Gumieniczek, A., Kozak, I., Żmudzki, P. and Hubicka, U., LC-UV and UPLCMS/MS Methods for analytical study on degradation of three antihistaminic drugs, ketotifen, epinastine and emedastine: Percentage degradation, degradation kinetics and degradation pathways at different pH. Processes, 2020;9(1):64-9. https://doi.org/10.3390/pr9010064

13. Joshi VK, Joshi A, Dhiman KS. The Ayurvedic Pharmacopoeia of India, development and perspectives. Journal of ethnopharmacology. 2017 Feb 2;197:32-8. https://doi.org/10.1016/j.jep.2016.07.030

14. Beckett AH, Stenlake JB, editors. Practical pharmaceutical chemistry: Part II fourth edition. A&C Black; 1988. https://zenodo.org/record/8319649

15. Vetrichelvan T, Prabakaran S. New Spectrophotometric Methods for the Determination of Racecadotril in Bulk Drug and Capsules. Indian Journal of Pharmaceutical Sciences. 2007 Mar 1;69(2):13-22. doi 10.4103/0250-474X.33168

16. Corradini D, Eksteen E, Eksteen R, Schoenmakers P, Miller N. Handbook of HPLC. CRC Press; 1998 Aug 21 https://doi.org/10.1201/9780203909751.

17. J. Mendham, R.C. Denney, J.D. Barnes, M. Thomas, B. Sivasankar, Textbook of Quantitative chemical analysis, Pearson publication, 2020. Page No.383-388. https://zenodo.org/record/8319649

18. Windholz M, Budavari S, Blumetti RF, Otterbein ES. The Merck Index. Merck & Co. Inc., Rahway, NJ. 1983;13:1051. DOI: 10.4236/als.2019.74012

19. World Health Organization. Guidelines on recreational water quality. Volume 1: coastal and fresh waters. World Health Organization; 2021 Jul 12.

20. Elmasry, M.S., Blagbrough, I.S., Rowan, M.G., Saleh, H.M., Kheir, A.A. and Rogers, P.J., Quantitative HPLC analysis of mebeverine, mesalazine, sulphasalazine and dispersible aspirin stored in a Venalink monitored dosage system with co-prescribed medicines. Journal of pharmaceutical and biomedical analysis, 2011;54(4):646-652.

21. Banda, J., Lakshmanan, R., Katepalli, R.B., Venati, U.K.R., Koppula, R. and Prasad, V.S., 2016. Determination of miconazole, a low bioavailability metabolite in human plasma by UHPLC–MS/MS: Application to a pharmacokinetic study. Journal of Chromatography B, 1008, pp.1-10.

22. Deepika Sharma, Komal Gupta, Pooja Chawla, Method Development and Validation for simultaneous estimation of Clotrimazole, Miconazole and Tinidazole by Reversed- Phase High Performance Chromatography Method in Tablets. Asian Journal of Pharmaceuticals and Clinical research, 2019;12:234-9.

23. Kavita Chandramore, Sandeep Sonawane, Development and Validation of Force Degradation Studies with Stability Indicating Method. 2020.

24. Connors KA., A Text book of Pharmaceutical Analysis; 3rd Edn; WileyInter science Publication, 2002, pp173-214.

25. Kats, M., Forced degradation studies: regulatory considerations and implementation. 2005.

26. Breaux, J., Jones, K. and Boulas, P., 2003. Analytical methods development and validation. Pharm. Technol, 1, pp.6-13.

27. Junod, S., 2008. FDA and clinical drug trials: a short history. FDLI Update, p.55.

28. Turkovic, V., Engmann, S., Egbe, D.A., Himmerlich, M., Krischok, S., Gobsch, G. and Hoppe, H., Multiple stress degradation analysis of the active layer in organic photovoltaics. Solar energy materials and solar cells, 2014;120:654-668.

29. ICH Guideline Q2R1 (Good Manufacturing Practice Guide for Active Pharmaceutical Ingredients) November 2000.

30. FDA, Guidance for Industry, Analytical Procedures and Methods Validation (Draft guidance), Food & Drug Administration, Rockville, US Department of Health and Human Services, 2000.

31. Polawar AR and Damle MC, Development and Validation of RP-HPLC method for estimation of Lurasidone Hydrochloride in Bulk and Pharmaceutical Dosage form. International Journal of Research in Pharmacy and Chemistry. 2014;4(2):327–332.