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MARALIXIBAT IN RAT PLASMA AND ITS PHARMACOLOGICAL STUDIES (BIO-ANALYTICAL METHOD DEVELOPMENT AND VALIDATION BY LC-MS)

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ABSTRACT

The bio-analytical method development of Maralixibat using Elobixibat as an internal standard is a convenient, fast, accurate, and consistent new LC-MS technique and was validated. The present work explains the development of the LC-MS/MS bio-analytical method by RP-18(150x4.6 mm, 3.5µ) column and a binary mixture(0.1% formic acid & methanol) of organic mobile phase in the ratio 60:40. By using liquid-liquid extraction process, these drugs are removed from rat plasma. The linearity in the standard curve was observed under the experimental concentration range**.** 10%-200% (6-12ng/ml) of Maralixibat. The calibration plots were linear with a regression coefficient of R^2> 0.999. Precision, Accuracy, Stability results, and Matrix effect were observed within the acceptable limit. The method is more accessible and effective for analyzing the sample in the body fluids. The work represents that Specificity, Suitability, Accuracy, and linearity parameters ideally agree with the USFDA guidelines and are efficiently practiced in rat plasma for pharmacokinetic studies

Keywords: Validation, Method development, Rat plasma, Maralixibat, LC-MS/MS.

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INTRODUCTION

For people with Alagille syndrome, a medication used to cure Cholestatic Pruritus, i.e., Maralixibat, has the brand name Livmarli.[1-4] It is a bile acid transporter(IBAT) inhibitor named Maralixibat chloride.[5,6] Maralixibat is an orally accessible inhibitor of the ileal bile salt transporter used to treat severe pruritus in people with cholestatic liver disease, particularly Alagille syndrome without Cirrhosis.[11-13] To treat Severe Pruritus, Alagille syndrome without Cirrhosis, and Cholestatic liver disease, Maralixibat is the only used inhibitor of ileal bile salt transporter. It is linked with fluctuations in transient serum enzyme, i.e., with long-term therapy and not with liver injury with Jaundice which is clinically apparent.[14,15] However, it has limited experience with its use. The European Medicines Agency (EMA) is the committee for human use of medicinal products (CHMP) is having positive thinking and recommended Livmaril with authorization from the market under atypical conditions for treating Alagille Syndrome(ALGS) with cholestatic Pruritus in patients. It is also known as (SHP625, LUM001, and lopixibat) and is a transporter inhibitor of ileal bile acid [odevixibat]. In patients with Alagille syndrome, Maralixibat treats Cholestatic Pruritus in one-year-old children. Previously, patients were treated with antihistamines alone or in combination [rifampin], [ursodeoxycholic acid], [cholestyramine], [naltrexone], and [sertraline] those who are suffering from cholestatic Pruritus associated with Alagille syndrome.[16,17] Based on the prescriber's clinical experience, the treatments given to treat Cholestatic Pruritus to assess its efficacy; are no clinical trials—partial external bile diversion and ileal used for treating surgical interventions. Maralixibat was the first FDA-approved drug for treating Cholestatic Pruritus in patients with Alagille syndrome. The present article is about the bioanalytical method development and validation. Commonly used sample preparation techniques and the main principles of Method validation will explain quantification, LC-MS/MS. In this article, we are focusing mainly on small molecule quantification. Until today, no method is available to quantify Maralixibat in any biological matrix. Pharmacokinetic studies in healthy rats are taking on Maralixibat for the first time.

EXPERIMENTAL

HPLC marked Acetonitrile, Formic acid, methanol, and water were bought from Merck (India) Ltd, Worli, Mumbai, India. All Active Pharmaceutical Ingredients (APIs) of Maralixibat and Elobixibat as standard references were bought from Cadila Health Care Limited, Ahmadabad. SCIEX QTRAP 5500 mass spectrometer was coupled to Waters alliance e-2695 model HPLC system with an electrospray ionization (ESI) interface.[18, 19] The chromatogram data was interpreted using The SCIEX software.[20-22] For separation and validation, the Waters X-bridge C18 column was used.

Chromatographic and Pharmacological Studies

Formic acid was taken as a mobile phase and methanol at 60:40 v/v with 1.0 mL/min(flow rate). The rate of injection volume and validation flow rate were 10µl and 5 minutes, respectively. The six active rats (app. 250g) were taken from Biological E Limited, Hyderabad, India, for the In vivo pharmacokinetic studies. The animal ethics committee approved the animal study protocol (Reg.No:1074/ PO /Re/S/05/CPCSEA).

Preparation of Internal Control Samples and Standard Sample

Six mg Maralixibat standard was taken in a 100 ml standard flask, added to the mark with the diluents, and sonicated for 10 minutes for complete dissolution. The solution was further diluted by taking In a 10 mL standard flask, the 1mL of the above solution was taken and the diluent was added up to the mark. Then, 0.4 mL solution is taken in a 10 mL standard flask from the above solution and filled up to the mark diluent. 5 mg of an internal standard of Elobixibat was added in a 10mL volumetric flask; diluent was added up to the mark and sonicated for 10 minutes for complete dissolution. In a 10mL standard flask, 0.4 mL of this solution was taken, and diluent was added to make up for the mark. Again in a 10 ml standard flask, 0.1 ml of this solution was taken diluent and added to the mark. 500µL of the standard stock solution was taken in a centrifuge tube of capacity 2 mL, and a mixture of 200µL of plasma and 300µL of methanol followed by 500µL internal standardand500µL of diluent was added and centrifuged for 20 minutes. The supernatant liquid is filtered and transferred into an HPLC vial.

Bio-Analytical Method Validation

The methods used for validation were linearity, precision, accuracy, sensitivity, selection, recovery study, matrix condition, reproducibility, stability, and reinjection.[23-31]

Selectivity, Matrix Effect, Accuracy, Precision, Recovery and Dilution Integrity

Evaluated by assessing six lots of individual rat plasma samples, and interference was checked and analyzed based on the retention time. Comparative effects of the Maralixibat matrix in connection with the ratio of height area of six different drug-free samples were studied. The control samples were resolute by replication analysis at a High-quality control(HQC), Medium quality control (MQC), lower limit of quantification (LLOQ), and low-quality control (LQC) internal. Except for LLOQ, i.e., 20% half of CV will be less than 15%, and 15% should be the accuracy. The six samples were analyzed and reproduced at each internal control concentration by extorting Maralixibat. Recovery determines the distinction between the extracted standard height ranges from the non-extracted standard height ranges. The matrix spiking explains dilution integrity with a concentrated analyte above the ULOQC.

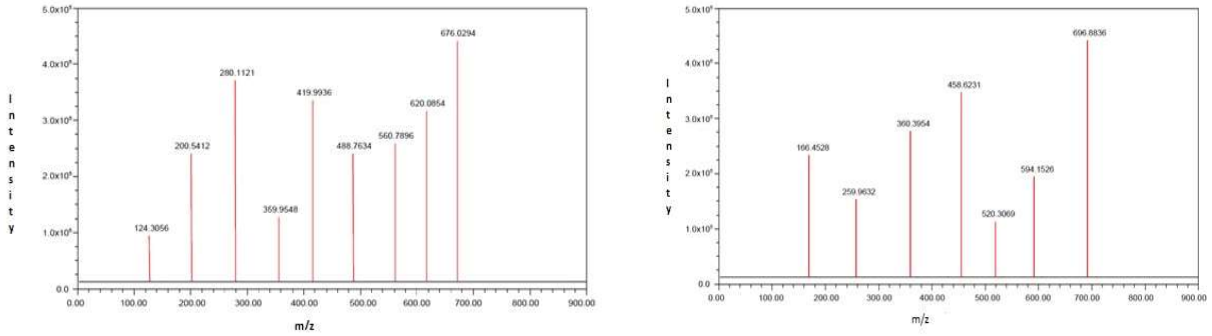
Stability and Pharmacokinetic Study

Comparing the stock solution and the newly prepared stock sample evaluates the stability of the stock solution. Six replicates at each level, the LQC and HQC concentration levels sample stability studies in plasma. As per USFDA guidelines, the analyte is stable if the change is equal to or less than 15%. At room temperature after 24 h, the stability of spiked rat plasma was acquired and studied. The LQC, MQC, and HQC (autosampler stability)of the extracted plasma samples were determined immediately after injection. After 12 or 18 hours, with wet extract stability at 2-8°C, the samples were re-injected. The reproducibility of reinjection was determined from the extracted plasma sample by comparing it with the injected one, immediately with samples stored in dry extract stability for 12 h and 18 h at -20±30°C. The freeze-thaw stability of freshly spiked internal control samples was evaluated, frosted at -31°C, and defrosted thrice. The long-term stability was evaluated by comparing the concentration with the initial concentrations and the concentration obtained after 24 h. The animals were famished for a night and drinking to thirst before experimenting. Under fasting conditions, the samples were injected into each rat[32,33] for Maralixibat

formulations and pharmacokinetic evaluation. The blood samples were collected after oral administration[34,35] of Maralixibat using a needle of 5/8 inch, 25-guage by clipping with a paper clip to the marginal ear vein of 0.5 mL to 1.0 mL volume at 0.25, 0.5, 0.75, 1.0, 1.25, 1.5, 1.75 h. The blood centrifugation was done at 2-8ºC temperature for 30 min at 5000rpm till the completion of the analysis. The supernatant plasma, i.e., clear, was procured and preserved at -30°C. The drug content in plasma samples was extracted using the liquid-liquid phase method and processed using a defined analytical method. The animals were returned to the shelter after the study for rehabilitation.

RESULTS AND DISCUSSION

Due to electron spray ionization on air pressure, the chemical ionization mode is maximum, which was chosen in this process. A mobile phase flow of 1mL/min was maintained to offer signal stability and sensitivity with the continuous flow to electron spray was highly responsive in the positive ion mode. The mass spectra of Maralixibat and Elobixibat are in Fig.-1.



(A) (B)

Fig.-1: Mass Spectra of (A) Maralixibat and (B) Elobixibat

Specificity and Matrix Effect

It was proved as a specific method for Maralixibat. In Fig.-2 and 3, blank and standard chromatograms are in acceptance. No Interference peaks were observed in standard and blank rat plasma chromatograms. The RSD percent for enhancement/ion suppression within the signal was 1% in LCMS for Maralixibat. The analyte's matrix effect[36,37] reveals that it was in the appropriate scope of ionization. In the matrix effect of Maralixibat, 98.46 and 98.55 were LQC and HQC. CV% of Maralixibat at LQC level were 1.77, 0.52. From these values, it was clear that analyte ionization on the matrix effect was present in the appropriate limit.

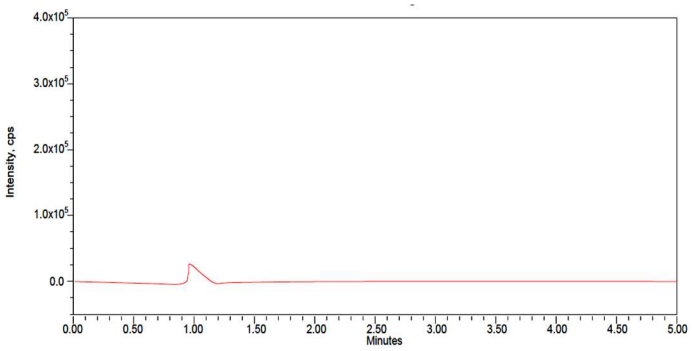


Fig.-2: Chromatogram of Blank

Linearity Precision and Accuracy

To the concentration, the calibration standards of the peak area ratio were proportional. The Maralixibat concentration range is 6-120 ng/mL. Table -1 shows the Linearity results[38] of Maralixibat, and Fig.-4 shows the calibration plot. Linear calibration plots were obtained and the Correlation coefficient for Maralixibat was found to be 0.999. We have calculated two parameters considering different internal control samples by combining all individual test results.[39] Based on the results obtained, the design was practical and precise. Table-2 shows the results for the precision of Maralixibat. The results for the precision of Maralixibat in the quality control sample were 98.51-99.92.

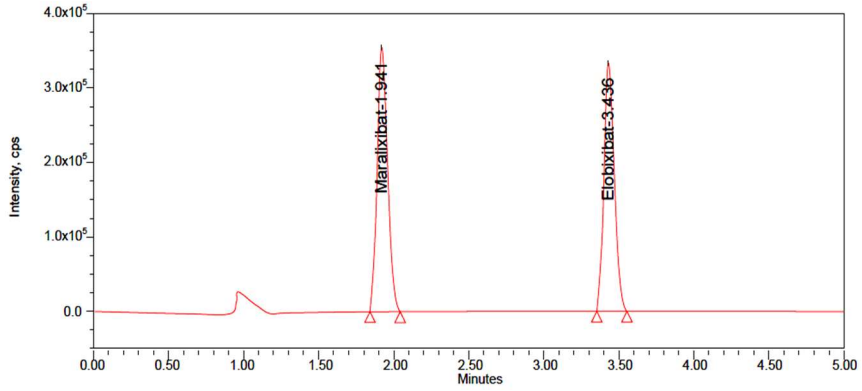


Fig.-3: Chromatogram of the Standard

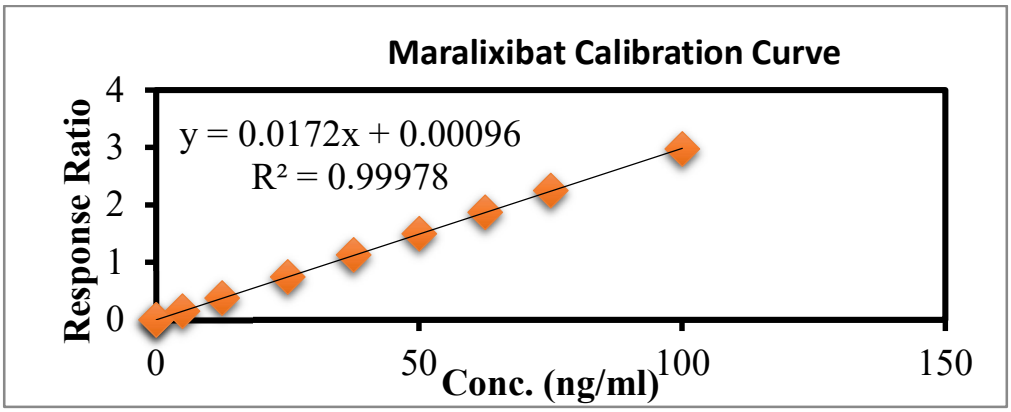


Fig.-4: Maralixibat Calibration Curve

Table-1: Linearity Results

Linearity	Maralixibat	
	Concentration.(ng/ml)	A response ratio of the area
1	6.00	0.356
2	15.00	0.875
3	30.00	1.754
4	45.00	2.537
5	60.00	3.554
6	75.00	4.251
7	90.00	5.253
8	120.00	7.005
	Slope	0.0172
	Intercept	0.00096
	CC	0.99978

Table-2: Accuracy and Precision of Maralixibat

Acquisition Batch ID	HQC	MQC	LQC	LLQC
	Nominal Concentration (ng/ml)			
	90	60	30	6
	Analyte peak area			
	$5.280x10^5$	$3.561x10^5$	$1.740x10^5$	$0.369x10^5$
	$5.251x10^5$	$3.561x10^5$	$1.796x10^5$	$0.365x10^5$
	$5.280x10^5$	$3.587x10^5$	$1.784x10^5$	$0.389x10^5$
	$5.282x10^5$	$3.597x10^5$	$1.790x10^5$	$0.367x10^5$
	$5.231x10^5$	$3.520x10^5$	$1.778x10^5$	$0.334x10^5$
	$5.250x10^5$	$3.523x10^5$	$1.735x10^5$	$0.300x10^5$
N	6	6	6	6

Mean	$5.262x10^5$	$3.558x10^5$	$1.771x10^5$	$0.354x10^5$
Standard Deviation	0.02132	0. 03178	0.02630	0.0318
% of CV	0.41	0.89	1.49	8.98
% of Mean Accuracy	98.51%	99.92%	99.47%	99.41%

mean±SD (n=6)

Recovery

The quantification method has acceptable efficiency in extraction but is not in connection with the recovery concentration. Recoveries of Maralixibat (98.16% - 105.14%) at MQC, LQC,% CV, and HQC levels ranged from 1.5-2.03 to Maralixibat; Thus, from the results, it is concluded that the method of quantification has good extraction efficiency.[83.97% - 100.06%]

Ruggedness

Two analysts resolved the recoveries percent and CV percent of Maralixibat on two columns of acceptable criteria for MQC, LQC, and HQC sample levels. These results prove Ruggedness. Recoveries percentage ranges between 98.55-100.00%, and CV% was between 0.39 – 1.84 to Maralixibat, respectively.

Auto Sampler Carryover and Stability

After completing sequential injections of ULOQC and LLQC at Maralixibat retention time, within the blank samples of plasma in rats, no response was observed in the peak region. The diluents for solution stability analysis and Maralixibat solutions were prepared and kept at 2-8ºC in a refrigerator. Stock solutions prepared within 24 hours are associated with fresh stock solutions. In the auto sample, the plasma stability of the bench top and auto sample was stable for 24h at 20ºC. From future stability, it was apparent that Maralixibat at -30ºc for 24h was stable at this storage temperature. Table-3 shows the results of the stability of Maralixibat.

***In-vivo* Pharmacokinetic Estimation**

The concentration-time profile of Maralixibat in rat plasma is shown in Fig.-5. In the case of the experimental formulation, the graph shows a bell-shaped curve. The pharmacokinetic parameters data are in Table-4. Maralixibat Cmax was found to be 56.5 ng/mL. The T_{max} for Maralixibat is 0.75h. The $T_{½}$ value was 1.5h. The AUC0-t for Maralixibat[40,41] is 59 ng/mL. Table-5 shows the pharmacokinetic results.

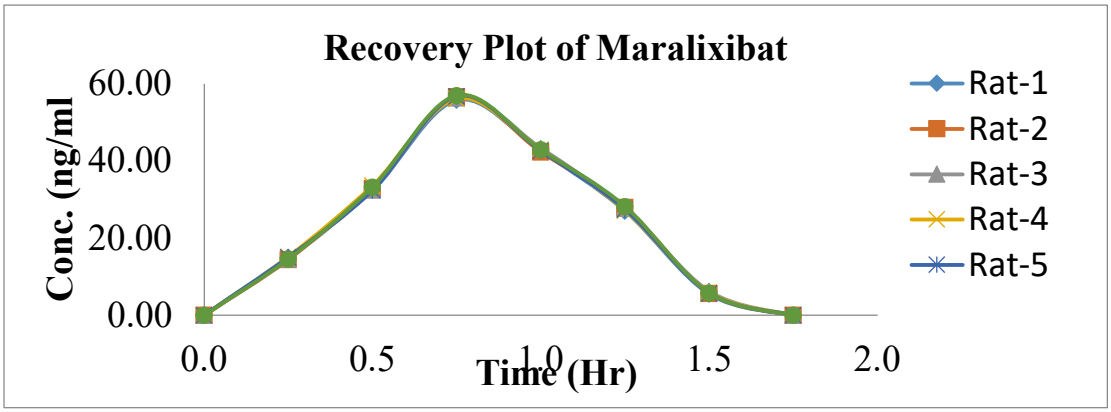


Fig.-5: Recovery Plot Maralixibat

Table-3: Stability Results of Maralixibat

Spiked plasma Stability experiment		Conc.(n=6,ng/ml)	% CV	%Accuracy
Benchtop stability	LQC	30	1.84	98.4
	MQC	60	0.93	100.06
	HQC	90	0.39	97.99
Autosampler stability	LQC	30	1.43	98.46
	MQC	60	0.87	99.75
	HQC	90	0.61	98.29
	LQC	30	0.19	83.97

Long term(Day28) stability	MQC	60	0.09	87.19
	HQC	90	0.44	89.15
Wet extract stability	LQC	30	1.62	98.57
	MQC	60	0.84	99.97
	HQC	90	0.56	98.77
Dry extract stability	LQC	30	1.68	98.06
	MQC	60	0.73	100.34
	HQC	90	0.42	98.29
Freeze thaw stability	LQC	30	1.4	99.13
	MQC	60	0.83	100.00
	HQC	90	0.44	98.38
Short term stability	LQC	30	0.18	95.76
	MQC	60	0.07	96.19
	HQC	90	0.03	97.07

mean±SD (n=6)

Table-4: Maralixibat Pharmacokinetic Parameters

Pharmacokinetic parameters	Maralixibat
AUC_{0-t}	59 ng-hr/ml
C_{max}	56.4 ng/ml
$AUC_{0-\infty}$	59 ng-hr/ml
t_{max}	0.8 hr
$T_{1/2}$	1.5 hr

CONCLUSION

The present HPLC-ESI-LCMS for determining Maralixibat in Rat plasma is very sensitive, selective, simple, and rapid. This technique was created and verified for the first time in rat plasma for the Maralixibat. The developed method was a fast, reproducible, rugged bioanalytical method. This method follows USFDA guidelines. The developed method was simple and efficient for determining the analyte of interest in body fluids through pharmacokinetic studies.

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CONFLICT OF INTERESTS

There were no conflicts of interest, according to the authors.

AUTHOR CONTRIBUTIONS

All the authors contributed significantly to this manuscript, participated in reviewing/editing and approved the final draft for publication. The research profile of the authors can be verified from their ORCID ids, given below:

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REFERENCES

1. B.M.Kamath, A. Baker, R. Houwen, L. Todorova and N. Kerkar, *Journal of Pediatric Gastroenterology and Nutrition*, **67,** 148(2018), https://doi.org/10.1097/MPG.0000000000001958
2. M.L.Bissonnette,J.C. Lane and A. Chang, *Kidney International Reports*, **2,** 493(2017), https://doi.org/10.1016/j.ekir.2016.11.002

3. B.M.Kamath,R.C. Bauer, K.M. Loomes,G. Chao, J. Gerfen, A. Hutchinson, et al, *Journal of Medical Genetics,* **49(2)**,138(2012), https://doi.org/10.1136/jmedgenet-2011-100544
4. P.Vajro, L. Ferrante, G. Paolella, *Clinics and Research in Hepatology and Gastroenterology*, **36(3)**, 275(2012), https://doi.org/10.1016/j.clinre.2012.03.019
5. P. A. Dawson, *Handbook of Experimental Pharmacology*, **201(201)**,169(2011), https://doi.org/10.1039/9781788016414-00062
6. M. H. Wong, P. N. Rao, M. J. Pettenati, P.A. Dawson, *Genomics,* **33(3)**, 538(1996), https://doi.org/10.1006/geno.1996.0233
7. S. Erickson, Z. Nahmias, I. S. Rosman, B. S. Kim, *Dermatologic Clinics,* **36(3)**, 325(2018), https://doi.org10.1016/j.det.2018.02.014
8. S.Molkara, S. Sabourirad, K. Molooghi, *International Journal of Dermatology*, **59**, 30(2019), https://doi.org/10.1111/ijd.14587
9. G. Rinaldi, *Dermatology Practical & Conceptual,* **9 (2)**, 90(2019), https://doi.org/10.5826/dpc.0902a03
10. Thulaseedhar Alumuri, Karuna Sree Merugu, L A Amarababu Namburi, Aravınd Kurnool, Arunachalam SaravanaVadivu, Selvakumar Balasubramanian, *Journal of AOAC International*, **106(5)**, 1138(2023), https://doi.org/10.1093/jaoacint/qsad046
11. Kalyani Koganti, Namburi L. A. Amara Babu, Naga Raju Sattu, Koya Prabhakara Rao, *Biomedical Chromatography*, e5816(2023), https://doi.org/10.1002/bmc.5816
12. J.A.Udell, C.S. Wang, J. Tinmouth, J.M. FitzGerald, N.T. Ayas, D.L. Simel, D,L., et al., *Journal of the American Medical Association*, **307(8)**, 832(2012), https://doi.org/10.1016/j.ijsu.2013.03.001
13. U. Kartoun, K.E. Corey, T.G. Simon, H. Zheng, R. Aggarwal, K. Ng, S.Y. Shaw, *PLOS ONE*, **12(10)**, e0186301(2017), https://doi.org/10.1371/journal.pone.0186301
14. B.Gondal, A. Aronsohn, *Seminars in Interventional Radiology*, **33(4),** 253(2016), https://doi.org/10.1055/s-0036-1592331
15. R. Bassari, J. B. Koea, *World Journal of Gastroenterology*, **21(5)**,1404(2015), https://doi.org/10.3748/wjg.v21.i5.1404
16. H. E. Lee, I. K. Chang, Y. Lee, C.D. Kim, Y.J. Seo, J.H. Lee, M. Im, *Journal of the European Academy of Dermatology and Venereology*, **28(12),**1654(2014), https://doi.org/10.1111/jdv.12403
17. S.Church Diana, K. Church, Martin, *The World Allergy Organization Journal*, **4 (Suppl 3)**, S22(2011), https://doi.org/10.1186/1939-4551-4-s3-s22
18. P.Ramadevi, K. Rambabu, *International Journal of Research in Pharmaceutical Sciences*,**11(4),**7854(2021), https://doi.org/10.26452/ijrps.v11i4.4670
19. A. R. Cahaya, Y. Harahap, and T. A. Rahmania, *Journal of Chromatography. B, Analytical Technologies in the Biomedical and Life Sciences*, **1215**, 123547(2023), https://doi.org/10.1016/j.jchromb.2022.123547
20. D.Ramchandran, A. Kethipalli, M. Krishnamurthy, *Journal of Pharmaceutical Sciences and Research*, **12**, 381(2020), https://doi.org/10.22159/ijap.2021v13i5.42097
21. M.D.Naykode, D.A. Bhagwat, S.D. Jadhav, H.N.More, *Research Journal of Pharmacy and Technology*,**10(3)**,708(2017), https://doi.org/10.5958/0974-360x.2017.00133.0
22. M.Singh, M.Charde, R.Shukla, M.C.Rita, *Research Journal of Pharmacy and Technology*, **4**, 1219(2011), https://doi.org/10.7439/ijbar.v2i1.22
23. S. Malathi, N.Aruna Devi, *International Journal of Pharmacy and Pharmaceutical Sciences*, **12**, 68(2020), https://doi.org/10.1615/telecomradeng.v79.i15.20
24. D.Senthil Rajan, G.Muruganathan, K.Shivkumar, T.Ganesh, *International Journal of Current Pharmaceutical Research*, **12**,15(2020), https://doi.org/10.22159/ijcpr.2020v12i2.37480
25. P. Shanmugasundaram, S. K. Kamarapu, *Research Journal of Pharmacy and Technology*, **10(10)**, 3379(2017), https://doi.org/10.5958/0974-360x.2017.00601.1
26. S. Gomathy, S.T.Narenderan, S.N.Meyyanathan, B.Gowramma, *Critical Reviews*, **7**, 4785(2020), https://doi.org/10.31838/jcr.07.19.560
27. A. S. Kumar, J. Manidipa,V. Debnath, L.Seshagiri, N.Rao, D.Gowri Sankar, *Research Journal of Pharmacy and Technology*, **9**, 549(2016), https://doi.org/10.5958/0974-360x.2016.00104.9

28. Y.Malak, A.A. Al-Bathish, M.K.Gazy, El-Jamal, *International Journal of Pharmaceutical Sciences and Research*,**12**,83(2020), https://doi.org/10.22159/ijpps.2020v12i2.35415
29. M. Gadhvi, P.Bhandari, A.Suhagia, B.N.Desai, *Research Journal of Pharmacy and Technology*, **6**, 200(2013).
30. K. Prabhakara Rao, N.L.A. Amara Babu, K. Kalyani Koganti, Babji, K. Palakeeti, S.V.Srinivas, *SN Applied Sciences*, **3**, 321,(2021), https://doi.org/10.1007/s42452-021-04219-x
31. Y.I.F.Hasanah, Y.Harahap, H.Suryadi, *International Journal of Applied Pharmaceutics*, **13**,148(2021), https://doi.org/10.22159/ijap.2021v13i2.39590
32. S. Prasanthi, G. Himabindu, *International Jornal of Applied Pharmaceutics*, **14**, 95(2022), https://doi.org/10.22159/ijap.2022v14i3.44440
33. R. Syed, R. Kantipudi, *International Journal of Applied Pharmaceutics*,**13**,198(2021), https://doi.org/10.7324/japs.2021.1101015
34. Y. Subba Rao, S. R. Pavani, Y. Subba Rao, S.R. Mannam, *Journal of Pharmaceutical Sciences and Research*,**12**,375(2020).
35. T.Subrahmanyam, S.V. Anuradha, A. Ratna, Kumari Shetty, R. Kumari, *Current Pharmaceutical Analysis*,**18(3)**, 291(2022), https://doi.org/10.2174/1573412917666210302145711
36. V. M. K. Naveen, B.Veeraswami, G. Srinivasa Rao, *Journal of Pharmaceutical Sciences*, **11**, 2272(2020), https://doi.org/10.26452/ijrps.v11ispl4.4454
37. G. K. Kumari, R. Kantipudi, *Journal of Pharmaceutical Sciences and Research*, **13**, 134(2021), https://doi.org/10.26452/ijrps.v11ispl4.4454
38. A. Raziq, Syed Umer, *International Journal of Applied Pharmaceutics*, **13**,171(2021).
39. K. P. Rao, N. L. Babu, K. Koganti, B. Palakeeti, K.S.V. Srinivas, *SN Applied Sciences*, **3(3)**, 321 (2021), https://doi.org/10.1007/s42452-021-04219-x
40. S. Prasanthi, G. Himabindu, *YMERmerJournal,* **21(2)**, 71832(2022), https://doi.org/10.37896/ymer21.02/67
41. S. Prasanthi, G. Himabindu, *Journal of Pharmaceutical Research International*, **34**,1(2022), https://doi.org/10.9734/jpri/2022/v34i27a35988

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