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Research Article

BIOANALYTICAL APPROACH TO ENSITRELVIR ESTIMATION USING LIQUID CHROMATOGRAPHY-TANDEM MASS SPECTROMETRY AND ITS APPLICATION TO PHARMACEUTICAL RESEARCH

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ABSTRACT

Objective: For the bioanalytical approach of ensitrelvir, a liquid chromatography-tandem mass spectrometry (LC-MS/MS) methodology was developed. This technique is simple to use, rapid, exact, active, and repeatable, and it uses remdesivir as an internal standard.

Methods: A phenyl column (250×4.6 mm, 5µ) and an organic mobile phase consisting of 0.1% trifluoroacetic acid and acetonitrile in a 50:50 v/v ratio are used in this article to summarize the latest advancements in bioanalytical LC-MS/MS procedures.

Results: An excellent linear concentration range from 3 ng/mL to 120 ng/mL was analyzed for ensitrelvir within 5 min (r^2 = 0.9998±0.005). It was determined that the outcomes for accuracy, precision, recovery, matrix effect, and stability were all within acceptable ranges.

Conclusion: The application successfully applies all the required criteria for pharmacokinetic investigations in rats, including system appropriateness, specificity, linearity, and accuracy, in accordance with US Food and Drug Administration requirements.

Keywords: Ensitrelvir, Remdesivir, Liquid chromatography-tandem mass spectrometry, USFDA guidelines, Rat plasma.

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INTRODUCTION

The clinical trial NCT05605093 (STRIVE: Shionogi Protease Inhibitor [Ensitrelvir]) is now studying the efficacy of ensitrelvir in the treatment of respiratory infections [1,2] and viral emergencies. One antiviral drug [3,4] that has been effective in treating COVID-19 [5,6] is ensitrelvir, which is marketed under the trade name Xocova. Its IUPAC name is 1-(2,4,5-Trifluorobenzyl)-3-[(1-methyl-1H-1,2,4-triazol-3-yl)methyl]-(6E)-6-[(6-chloro-2-methyl-2H-indazol-5-yl)imino]-1,3,5-triazinane-2,4-dione. It is an orally active 3C-like protease inhibitor [7-9] that Shionogi and Hokkaido University developed together. Orally administered. Temporary drops in HDL [10,11] and spikes in blood triglycerides [12,13] are the most often reported side effects. When it comes to treating COVID-19-related loss of smell and taste, ensitrelvir has some promise. A trial conducted in 2023 found that the medication reduced these symptoms [14] by 39%.

There is currently no way to bio-analyze ensitrelvir in a biological matrix. This research set out to find a way to measure ensitrelvir in rat plasma more quickly and accurately than previous methods using remdesivir as an internal standard and a novel liquid chromatography-tandem mass spectrometry (LC-MS/MS) technique.

METHODS

Chemicals and reagents

We bought high-performance liquid chromatography (HPLC)-grade acetonitrile (ACN), trifluoroacetic acid, and water from Merck (India) Ltd. in Worli, Mumbai, India. Zydus Cadila Healthcare Ltd. of Ahmedabad supplied remdesivir, and Jubilant Biosys Ltd. of Karnataka supplied ensitrelvir API.

Equipment

We utilized a QTRAP 5500 triple quadrupole mass spectrometer in conjunction with Waters Alliance E2695 type HPLC equipment. The process was carried out using the ABSCIEX software [15-17].

Pharmacokinetic study

Selection of animals

Six white New Zealand rats, each weighing around 250 g, were procured from Biological E Limited in Hyderabad, India, for the purpose of conducting *in vivo* pharmacokinetic investigations. An animal ethics committee at the institution gave its stamp of approval to the research protocol (Reg.No:1074/PO/Re/S/26/CPCSEA).

Chromatographic conditions

Isocratic mode was used to administer the chromatographic separation at room temperature using phenyl columns (250×4.6 mm, 5 µ. At a flow rate of 1.0 mL/min, a mobile phase mixture was used, consisting of 50/50 v/v of ACN and 0.1% tri fluoro acetic acid. The dosage rate was 10 µl, and the duration of the run was 5 min. The patient's MS status is shown in Table 1.

Preparation of standard and internal control samples

Preparation of ensitrelvir stock solution

In a 100 mL volumetric flask, combine 6 mg of the ensitrelvir working standard with 70 mL of diluents. Sonicate for 10 min to fully dissolve the contents, and then add diluent until the flask is filled to the mark. Reduced the volume from 0.4 mL to 10 mL using a diluent. Add 1 mL to a 10 mL volumetric flask for further diluting.

Table 1: MS conditions

Ion mode	Positive
Collision gas	nitrogen
Collision energy	14 V
Ion spray voltage	5500 V
Entrance potential	10 V
Exit potential	7 V
Declustering potential	40 V
Dwell time	1 s
Source temperature	550°C
Drying gas temperature	250°C
Cone gas flow	50 L/h
Drying gas flow stream	5 mL/min

Preparation of internal standard (remdesivir) stock solution (240 ng/mL)

Transfer 6 mg of remdesivir working standard, weighed, to a 100 mL volumetric flask that has been diluted with diluent to volume. Adjust the volume from 0.4 mL to 10 mL by adding diluent. Add 1 mL of the aforementioned solution to a 10-mL volumetric flask and fill it up with diluents until it reaches the mark.

Preparation of standard solution

In a 2 mL centrifuge tube, 200 µL of plasma and 300 µL of ACN were used for standard preparation. Then, 500 µL of standard stock solutions, 500 µL of IS, and 500 µL of diluents were added, and the mixture was vortexed for 10 min. Then, for 20 min, these samples were centrifuged at 4000 rpm. The solution was filtered through a 0.45 µ nylon syringe filter and then transferred to a vial before being injected into a system.

Bio-analytical method validation

Matrix condition, stability, sensitivity, linearity, accuracy, and precision were among the areas where the approach was verified [18-26].

Selectivity

We checked for interference at the retention time selectivity by analyzing plasma samples from six distinct rats.

Matrix effect

Obtaining the matrix effect from six different drug-free plasma samples of ensitrelvir was achieved by comparing their height-area ratios. Six separate plasma lots were used in triplicate experiments conducted at medium quality control (MQC) levels with an acceptable accuracy of <15%.

Precision and accuracy

The information was derived from internal control samples that were analyzed at four different quality control levels: Low-quality control (LQC), MQC, high-quality control (HQC), and lower limit of quality control. The accuracy should be within 15%, and the coefficient of variance (CV) should be <15%, with the exception of LLOQ, where the CV should be 20%.

Recovery

Using ensitrelvir extraction, six replicate samples were analyzed at each internal control concentration. One way to measure recovery is to compare the height areas of the extracted and unextracted standards [27].

Carry over

Carryover is discussed in references [28,29] and refers to the analyte that remains in the chromatographic system after diluting the sample with a blank matrix while the analyte concentration is above the upper limit of quality control (ULOQC).

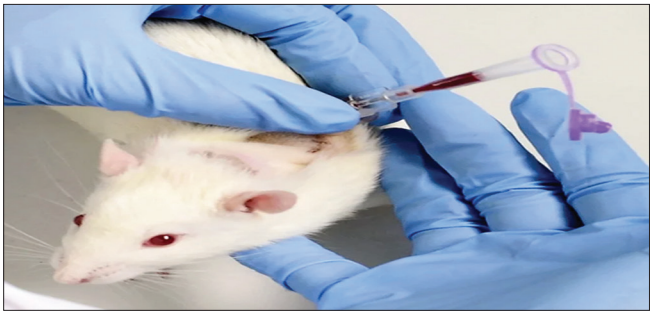

Fig. 1: Sampling of rat

Dilution integrity

To demonstrate dilution integrity, it is necessary to spike the matrix with an analyte concentration greater than the ULOQC and then dilute the sample with a blank matrix [30].

Stability

The stability sample and the sample from the new stock sample preparation are compared to the act of stock solution stability [31]. Six replicates were used for each concentration level in the plasma sample stability experiments conducted with LQC and HQC. According to US Food and Drug Administration (USFDA) rules [32], analyte stability was defined as a change of <15%. We tested the integrity of spiking rat plasma that had been kept at room temperature for 24 h. After being kept at room temperature in an autosampler for 24 h, the stability of spiked rat plasma was assessed. Using wet extract stability at room temperature after 12 h and 18 h at 2–8°C, we compared the autosampler stability (LQC, MQC, and HQC) of freshly injected plasma extract samples with those of reinjected samples. The test for reinjection repeatability included comparing plasma samples that were extracted and injected right away with those that were reinjected after being stored in the dry extract stability at room temperature for 12 h and 18 h at −20°±3°C. The stability of the samples was tested by comparing them to newly spiked internal control samples and steadiness samples that had been frozen at −31°C and thawed 3 times. The short-term stability test lasted for 7 days at 7°C. The initial concentration was compared to the concentrations obtained after 24 h to evaluate the stability over the long term.

Pharmacokinetic study

All animals are given water freely and then deprived overnight before the trial. An over-the-counter anesthetic method was used. The ensitrelvir standard underwent pharmacokinetic assessment. All of the rats were given the standard when they were fasting. At 1, 2, 3, 5, 10, 20, 30, 40, and 50 h after oral administration of ensitrelvir, rats' marginal ear veins were pricked with a paper clip to expose the veins, and a 25-gauge, 5/8-inch needle was used to draw 0.3 mL of blood (Fig. 1). A 10% ethylene diamine tetra acetic acid solution was added to the Eppendorf tubes used for blood collection. The blood was spun at 4000 rpm for 20 min in a temperature range of 2–8°C. We collected the clear supernatant plasma and kept it at −30°C until we could analyze it. We used a newly developed analytical approach to determine the drug concentration in the plasma samples after subjecting them to liquid–liquid phase extraction. The animals were taken back to the animal shelter for rehabilitation after the research.

Based on plasma concentration data, the pharmacokinetic characteristics for oral delivery of ensitrelvir were calculated. Pharmacokinetic characteristics such as area under the curve (AUC), maximum concentration (C_{max}), duration to achieve peak concentration (T_{max}), and the time at which C_{max} occurred: Starting at zero and continuing all the way to infinity on the concentration-time curve, the data were measured using the trapezoidal rule approach. From the

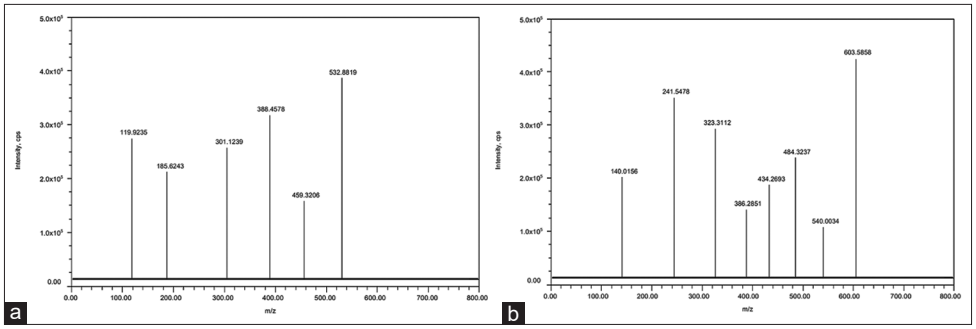

Fig. 2: Mass spectra of (a) ensitrelvir and (b) ritonavir (IS)

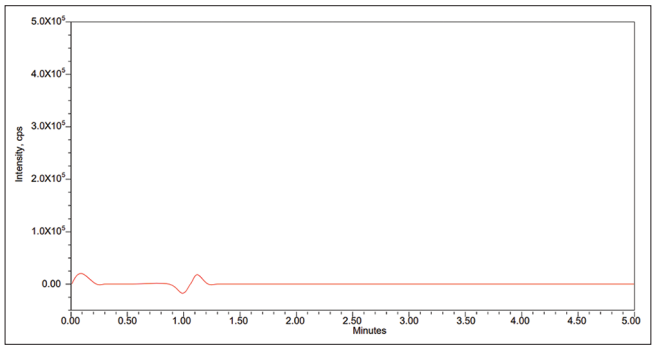

Fig. 3: Chromatogram of blank

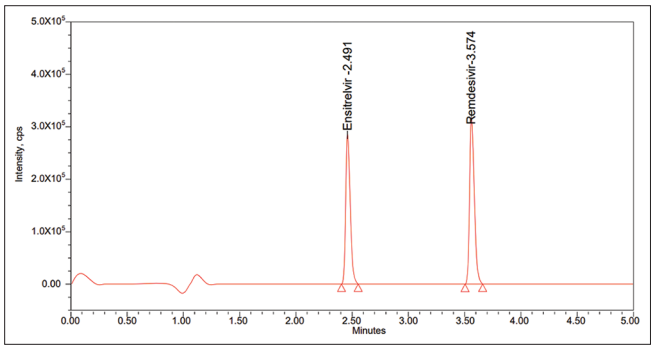

Fig. 4: Chromatogram of standard

graph, we were able to get C_{max} and T_{max}. The mean±Standard deviation (SD) is used to represent all values. (Mean–Dispersion).

RESULTS AND DISCUSSION

When using this method's chosen mode of air pressure chemical ionization, electrospray ionization yields the best result. The positive ion mode provides sensitivity and signal stability with continuous flow to the electrospray ion, and the 10 µL/min ensitrelvir mobile phase flow is quite sensitive in this mode. The mass spectra of remdesivir (IS) and ensitrelvir are shown in Fig. 2.

Specificity

The approach for researching ensitrelvir is shown to be particular. Figs. 3 and 4, and the chromatograms of the standard and blank samples. We noticed the chromatograms of both the standard and blank rat plasma, which did not have any interference peaks.

Matrix effect

Under these conditions, the matrix impact [33] on analyte ionization is within an acceptable range, as the percent relative SD for within-signal ion suppression/enhancement for ensitrelvir in LC-MS/MS was found to be 1.0%. The ensitrelvir matrix impact LQC and HQC were 96.54% and 97.78%, respectively. CV was 2.01 at the LQC level and 0.44 at the HQC level. It shows that the matrix influence on the analyte's ionization is within the acceptable range.

Linearity

Concentration had a direct correlation with the peak area ratio of the standards used for calibration. Ensitrelvir is effective at concentrations ranging from 3 to 120 ng/mL. Table 2 shows the linearity findings of ensitrelvir, and Fig. 5 shows its calibration plot [34]. With a correlation value of 0.9998, the calibration curve seemed to be linear.

Precision and accuracy

All of the test findings from the several internal control samples were combined to determine the accuracy and precision [35]. The facts presented here made it quite clear that this technique worked. You may see the ensitrelvir precision findings in Table 3. The accuracy of

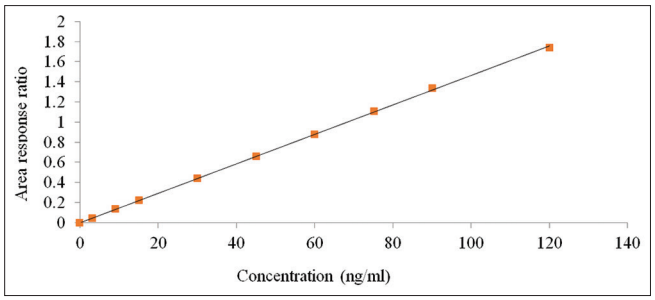

Fig. 5: Calibration plot of ensitrelvir

Table 2: Results of linearity

Linearity	Ensitrelvir	
	Conc.(ng/mL)	Area response ratio
1	3.00	0.045
2	9.00	0.133
3	15.00	0.220
4	30.00	0.439
5	45.00	0.657
6	60.00	0.881
7	75.00	1.107
8	90.00	1.336
9	120.00	1.744
Slope		0.0147
Intercept		0.00103
CC		0.99980

ensitrelvir in quality control samples ranges from 95.37 to 98.07. The ensitrelvir CV <5% of the total samples used for internal control.

Recovery

The findings showed that the bioanalytical approach had high extraction efficiency for ensitrelvir at LQC, MQC, and HQC levels. This further demonstrated that the recovery was unrelated to focus. At LQC,

Table 3: Precision and accuracy of ensitrelvir

S. No.	HQC	MQC	LQC	LLQC
	Nominal concentration (ng/mL)			
	90	60	9	3
	Analyte peak area			
1	4.188×10^5	2.801×10^5	0.411×10^5	0.138×10^5
2	4.172×10^5	2.784×10^5	0.417×10^5	0.144×10^5
3	4.196×10^5	2.793×10^5	0.406×10^5	0.129×10^5
4	4.198×10^5	2.806×10^5	0.413×10^5	0.137×10^5
5	4.215×10^5	2.781×10^5	0.401×10^5	0.124×10^5
6	4.203×10^5	2.815×10^5	0.415×10^5	0.141×10^5
n	6	6	6	6
Mean	4.195×10^5	2.797×10^5	0.411×10^5	0.136×10^5
SD	0.01450	0. 01313	0.00599	0.00756
% CV	0.35	0.47	1.46	5.58
% Accuracy	98.06	98.07	96.07	95.37

Mean±SD (n=6). SD: Standard deviation, LQC: Low-quality control, HQC: High-quality control, MQC: Medium-quality control

Table 4: Stability results of ensitrelvir

Stability experiment spiked plasma	Mean Area±SD	% CV	%Recovery
Benchtop stability			
LQC	$0.405\times10^5\pm0.00327$	0.81	94.67
MQC	$2.795\times10^5\pm0.00288$	0.10	98.00
HQC	$4.165\times10^5\pm0.00306$	0.07	97.36
Autosampler stability			
LQC	$0.410\times10^5\pm0.00669$	1.63	95.84
MQC	$2.775\times10^5\pm0.00789$	0.28	97.30
HQC	$4.201\times10^5\pm0.00808$	0.19	98.20
Long-term (day 28) stability			
LQC	$0.356\times10^5\pm0.00402$	1.13	83.22
MQC	$2.441\times10^5\pm0.00519$	0.21	85.59
HQC	$3.605\times10^5\pm0.00331$	0.09	84.27
Wet extract 18 h stability			
LQC	$0.404\times10^5\pm0.00280$	0.69	94.44
MQC	$2.788\times10^5\pm0.00561$	0.20	97.76
HQC	$4.175\times10^5\pm0.00306$	0.07	97.59
Dry extract 18 h stability			
LQC	$0.407\times10^5\pm0.00483$	1.19	95.14
MQC	$2.768\times10^5\pm0.00543$	0.20	97.05
HQC	$4.157\times10^5\pm0.00407$	0.10	97.17
Freeze thaw stability			
LQC	$0.404\times10^5\pm0.00237$	0.59	94.44
MQC	$2.797\times10^5\pm0.00437$	0.16	98.07
HQC	$4.154\times10^5\pm0.00294$	0.07	97.10
Short term stability			
LQC	$0.397\times10^5\pm0.00463$	1.17	92.80
MQC	$2.692\times10^5\pm0.00409$	0.15	94.39
HQC	$4.096\times10^5\pm0.00308$	0.08	95.75

Mean±SD (n=6). SD: Standard deviation, LQC: Low-quality control, HQC: High-quality control, MQC: Medium quality control

MQC, and HQC levels, the recoveries for ensitrelvir varied from 94.20% to 98.22%, and the percentage CV was between 0.25 and 1.54. Good extraction efficiency was shown by the findings of the bioanalytical approach.

Ruggedness

The ensitrelvir % recoveries and percent CV, as assessed by two separate analyzers using two separate columns, met the acceptable standards in the HQC, LQC, MQC, and LLQC samples. A method's ruggedness was shown by the findings. For ensitrelvir, the percentage recoveries varied between 98.61% and 98.06%. The percent CV values varied between 0.07 and 1.37. A method's ruggedness was shown by the findings.

Table 5: Pharmacokinetic parameters of ensitrelvir

Pharmacokinetic parameters	Ensitrelvir
AUC_{0-t}	1799 ng-h/mL
C_{max}	56.172 ng/mL
$AUC_{0-\infty}$	1799 ng-h/mL
t_{max}	3 h
$T_{1/2}$	40 h

$AUC_{0-\infty}$: Area under the curve extrapolated to infinity
AUC_{0-t}: Area under the curve up to the last sampling time
C_{max}: The maximum plasma concentration
T_{max}: The time to reach peak concentration, $T_{1/2}$: Time the drug concentration

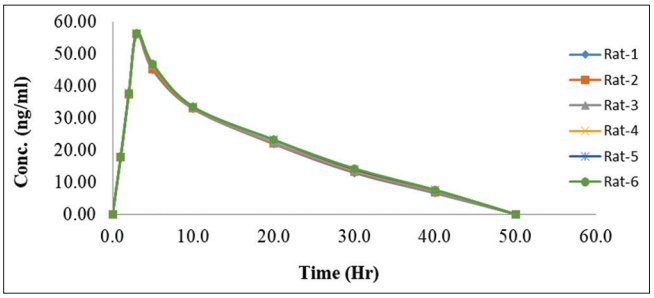

Fig. 6: Recovery plot of ensitrelvir

Auto sampler carryover

Following many injections of LLQC and ULQC at ensitrelvir retention periods, no peak area response of the drug was seen in the plasma samples of untreated rats. This approach does not display auto-sampler carryover.

Stability

A solution stability investigation was conducted by preparing ensitrelvir solutions with diluents and then placing them in a refrigerator at a temperature range of 2–8°C. Stock solutions that were produced 24 h before use paired with fresh stock solutions. After 24 h at room temperature and 24 h in the autosampler, the plasma stability of both the bench top and autosampler remained constant. It was determined through further stability testing that ensitrelvir may be stored at −30°C for up to 24 h without losing any of its efficacy. The findings of ensitrelvir's overall stability are shown in Table 4.

***In vivo* Pharmacokinetic Evaluation**

Fig. 6 shows the ensitrelvir plasma concentration-time curve in rats. It looked like a bell curve on the graph. It was shown that ensitrelvir remained detectable in the blood for 1.0 and 40 h after oral and intravenous treatment, respectively, suggesting that the drug release from the formulation was successful.

The results of the calculations of the pharmacokinetic parameters C_{max}, T_{max}, $T_{1/2}$, AUC_{0-t}, and $AUC_{0-\infty}$ are shown in Table 5. Ensitrelvir had a C_{max} of 56.172 ng/mL. An endpoint of 3.0 h was determined for the half-life of ensitrelvir. Ensitrelvir had a $t_{½}$ value of 40 h. It was determined that ensitrelvir had an AUC_{0-t} of 1799 ng-hr/mL. Table 5 displays the pharmacokinetic parameters.

CONCLUSION

The development and validation of a more sensitive HPLC-ESI-LCMS/MS technique for the detection of ensitrelvir in rat plasma was a first. This bioanalytical approach is tough, quick, and repeatable. Following USFDA requirements, this approach was verified. To see the studied analyte in bodily fluids and conduct pharmacokinetic research, a simple and effective technique was devised.

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AUTHORS CONTRIBUTION

All authors are contributed equally.

CONFLICTS OF INTEREST

The authors report no financial or any other conflicts of interest in this work.

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REFERENCES

1. Moriyama M, Hugentobler WJ, Iwasaki A. Seasonality of respiratory viral infections. Annu Rev Virol. 2020;7(1):83-101. doi: 10.1146/annurev-virology-012420-022445, PMID 32196426
2. Minozzi S, Pifferi S, Brazzi L, Pecoraro V, Montrucchio G, D'Amico R. Topical antibiotic prophylaxis to reduce respiratory tract infections and mortality in adults receiving mechanical ventilation. Cochrane Database Syst Rev. 2021;1(1):CD000022. doi: 10.1002/14651858.CD000022.pub4, PMID 33481250
3. Yin H, Jiang N, Shi W, Chi X, Liu S, Chen JL, *et al*. Development and effects of influenza antiviral drugs. Molecules. 2021;26(4):810. doi: 10.3390/molecules26040810, PMID 33557246
4. Bobrowski T, Melo-Filho CC, Korn D, Alves VM, Popov KI, Auerbach S, *et al*. Learning from history: Do not flatten the curve of antiviral research! Drug Discov Today. 2020;25(9):1604-13. doi: 10.1016/j.drudis.2020.07.008, PMID 32679173
5. McCarthy MW. Ensitrelvir as a potential treatment for COVID-19. Expert Opin Pharmacother. 2022;23(18):1995-8. doi: 10.1080/14656566.2022.2146493, PMID 36350029
6. Jiang X, Wang R. Wildlife trade is likely the source of SARS-CoV-2. Science. 2022;377(6609):925-6. doi: 10.1126/science.add8384, PMID 36007033
7. Unoh Y, Uehara S, Nakahara K, Nobori H, Yamatsu Y, Yamamoto S, *et al*. Discovery of S-217622, a noncovalent oral SARS-CoV-2 3CL protease inhibitor clinical candidate for treating COVID-19. J Med Chem. 2022;65(9):6499-512. doi: 10.1021/acs.jmedchem.2c00117, PMID 35352927
8. Kim Y, Lovell S, Tiew KC, Mandadapu SR, Alliston KR, Battaile KP, *et al*. Broad-spectrum antivirals against 3C or 3C-like proteases of picornaviruses, noroviruses, and coronaviruses. J Virol. 2012;86(21):11754-62. doi: 10.1128/JVI.01348-12, PMID 22915796
9. Uraki R, Kiso M, Iida S, Imai M, Takashita E, Kuroda M, *et al*. Characterization and antiviral susceptibility of SARS-CoV-2 Omicron BA.2. Nature. 2022;607(7917):119-27. doi: 10.1038/s41586-022-04856-1, PMID 35576972
10. Reyes-Soffer G, Ginsberg HN, Berglund L, Duell PB, Heffron SP, Kamstrup PR, *et al*. Lipoprotein(a): A genetically determined, causal, and prevalent risk factor for atherosclerotic cardiovascular disease: A scientific statement from the American Heart Association. Arterioscler Thromb Vasc Biol. 2022;42(1):e48-60. doi: 10.1161/ATV.0000000000000147, PMID 34647487
11. Pirillo A, Catapano AL, Norata GD. HDL in infectious diseases and sepsis. Handb Exp Pharmacol. 2015;224:483-508. doi: 10.1007/978-3-319-09665-0_15, PMID 25522999
12. Krisnangkura K. Estimation of heat of combustion of triglycerides and fatty acid methyl esters. J Am Oil Chem Soc. 1991;68(1):56-8. doi: 10.1007/BF02660311
13. Lok CM, Ward JP, Van Dorp DA. The synthesis of chiral glycerides starting from D-and L-serine. Chem Phys Lipids. 1976;16(2):115-22. doi: 10.1016/0009-3084(76)90003-7, PMID 1269065
14. Lenharo M. New pill helps COVID smell and taste loss fade quickly. Nature. 2023. doi: 10.1038/d41586-023-03244-7, PMID 37853192
15. Ramadevi P, Rambabu K. Bio analytical method development and validation for ezetimibe and Pitavastain and its applications to pharmacokinetic studies in Rat plasma by using LCMS/MS. Int J Res Pharm Sci. 2020;11:7854-62.
16. Eluru A. Surendra Babu K. Bio analytical method development and validation for aplidine in rat plasma and their pharmacokinetic studies by LCMS. World J Pharm Pharm Sci. 2019;8:1201-9.
17. Ramchandran D, Kethipalli A, Krishnamurthy M. Bio- and Cytrarabine in rat plasma by LC-MS/MS and its application in pharmacokinetic studies. J Pharm Sci Res. 2020;12:381-6.
18. Naykode MD, Bhagwat DA, Jadhav SD, More HN. Analytical and bio analytical method for quantification of pure azilsartan, not its salt by RP-HPLC. Res J Pharm Technol. 2017;10(3):708-14. doi: 10.5958/0974-360X.2017.00133.0
19. Singh M, Charde M, Shukla R, Rita MC. Determination of calcipotriene in calcipotriene cream 0.05% w/w by RP-HPLC method development and validation. Res J Pharm Technol. 2011;4:1219-23.
20. Malathi S, Arunadevi N. Development and validation of stability-indicating simultaneous estimation of metformin and alogliptin in tablets by high-performance thin layer chromatography. Int J Pharm Pharm Sci. 2020;12:68-73.
21. Senthil Rajan D, Muruganathan G, Shivkumar K, Ganesh T. Development and validation of HPLC method for simultaneous quantification of vasicine, glycyrrhizin and piperine in poly herbal cough syrup. Int J Curr Pharm Res. 2020;12:15-9.
22. Shanmugasundaram P, Kamarapu SK. RP-HPLC method for the simultaneous estimation and validation of amlodipine besylate and atenolol in bulk and tablet dosage form in biorelevant dissolution medium (Fassif). Res J Pharm Technol. 2017;10(10):3379-85. doi: 10.5958/0974-360X.2017.00601.1
23. Gomathy S, Narenderan ST, Meyyanathan SN, Gowramma B. Development and validation of HPLC method for the simultaneous estimation of apigenin and luteolin in commercial formulation. J Crit Rev. 2020;7:4785-90.
24. Kumar SA, Debnath A, Rao JV, Sankar DG. Development and validation of a sensitive RP-HPLC method for simultaneous estimation of rosuvastatin and fenofibrate in tablet dosage form by using PDA detector in Gradient mode. Res J Pharm Technol. 2016;9(5):549-54. doi: 10.5958/0974-360X.2016.00104.9
25. Malak Y, Al-Bathish AA, Gazy AA, El-Jamal MK. RP-HPLC and chemometric methods for the determination of two anti-diabetic mixtures;metformin hydrochloride-canagliflozin and metformin hydrochloride-gliclazide in their pharmaceutical formulation. Int J Pharm Pharm Sci. 2020;12:83-94.
26. Gadhvi MP, Bhandari A, Suhagia BN, Desai UH. Development and validation of RP-HPLC method for simultaneous estimation of atazanavir and ritonavir in their combined tablet dosage form. Res J Pharm Technol. 2013;6:200-3.
27. Prabhakara Rao K, Babu NL, Koganti K, Palakeeti B, Srinivas KS. Related substances method development and validation of an LCMS/MS method for quantification of selexipag and its related impurities in rat plasma and its application to pharmacokinetic studies. SN Appl Sci. 2021;3:321.
28. Chaitanya SM, Nissankararao S, Gandham SL. A sort of validated Bio analytical method developed for the estimation of etoposide and cisplatin in rat plasma by using two different advanced liquid chromatographic techniques like HPLC and UPLC and its application in bio equivalence studies. Int J Res Pharm Sci. 2021;12(1):708-17. doi: 10.26452/ijrps.v12i1.4167
29. Naveen VM, Veeraswami B, Srinivasa Rao G. High response bio-analytical validation approach of Nadolol and Bendroflumethiazide by LC-MS/MS on rat plasma. Int J Res Pharm Sci. 2020;11(SPL4):2272-9. doi: 10.26452/ijrps.v11iSPL4.4454
30. Kumari GK, Kantipudi R. Bio-analytical method development and validation for Avapritinib in rat plasma by LC-MS/MS. J Pharm Sci Res. 2021;13:134-7.
31. Hemanth Kumar AK, Sudha V, Vijayakumar A, Padmapriyadarsini C. Simultaneous method for the estimation of Bidaquiline and Delamanid in human plasma using high performance liquid chromatography. Int J Pharm Pharm Sci. 2021;13:36-40.
32. Hasanah YI, Harahap Y, Suryadi H. Development and validation method of cyclophosphamide and 4-hydroxy cyclophosphamide with 4-hydroxy cyclophosphamide-D_4 as internal standard in dried blood spots using UPLC-MS/MS. Int J Appl Pharm. 2021;13:148-52.
33. Pappula N, Kodali B, Datla PV. Selective and rapid determination of tadalafil and finasteride using solid phase extraction by high performance liquid chromatography and tandem mass spectrometry. J Pharm Biomed Anal. 2018;152:215-23. doi: 10.1016/j.jpba.2018.01.020, PMID 29427880
34. Priyadarshini I, Akila Devi D. Development and validation of an LC-MS/MS method for the determination of tenofovir and emtricitabine. Int J App Pharm. 2024;16:116-23. doi: 10.22159/ijap.2024v16i2.49667
35. Sentat T, Lucida H, Widyati W, Nasif H, Harahap Y, Harijono P, Ratih R. Development and validation of a bioanalytical method for therapeutic drug monitoring of amikacin in human plasma using ultra-performance liquid chromatography-tandem mass spectrometry. Int J Appl Pharm. 2024;16(Special Issue 1):140-4.