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Original Article

PERAMIVIR AND RELATED IMPURITIES IN RAT PLASMA AND ITS APPLICATIONS IN PHARMACOKINETIC STUDIES (BIOANALYTICAL METHOD DEVELOPMENT AND VALIDATION BY LC-MS/MS)

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ABSTRACT

Objective: New LC-MS/MS method for the estimation of Peramivir and its associated substances was developed and validated

Methods: Optimized (Developed) method includes gradient elution of peramivir and its related substances with a flow of 1 ml/min and waters X-bridge C_{18} column of dimensions 150 mmx4.6 mm, 3.5µ. 0.1% formic acid and acetonitrile were used as the mobile phase. Sarilumab was used as an internal standard. 40 min run time was used to separate peramivir and its related substances.

Results: The calibration curve was linear in the concentration percentage range from 10%-200% of Peramivir and its related substances. The calibration charts plotted were linear with a regression coefficient of R^2>0.999. Accuracy, precision, recovery, matrix effect and stability results were found to be within the suitable limits. A Simple and efficient method was developed and utilized in pharmacokinetic studies to see the investigated analyte in body fluids.

Conclusion: This application denotes all parameters such as accuracy, precision, recovery, stability etc, which are in good agreement with the USFDA guidelines and are effectively applied to the investigation of the pharmacokinetic studies in rat plasma.

Keywords: Peramivir, LC-MS/MS, Development, Validation, Rat plasma

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INTRODUCTION

Peramivir is a known analytical anti-viral drug [1, 2] usually treated in influenza [3, 4] related diseases, it has been developed by various Pharmaceutical companies. Peramivir is a neuraminidase inhibitor [5, 6] acting as a transition state analogue [7, 8] inhibitor of influenza neuraminidase, preventing viruses [9, 10] from emerging from infected cells. It has been approved for intravenous administration [11-13]. In the 2008-2009 intramuscular [14, 15] peramivir phase II seasonal influenza study, there was no effect for the primary end point of median improvement on the alleviation of symptoms in subjects with confirmed acute uncomplicated influenza infection versus placebo. On October 23rd, the US food and drug administration (FDA) [16] has issued an emergency use authorization for peramivir, allowing the use of an intravenous drug for hospitalized patients only in cases where other available treatment methods are ineffective or unavailable; for example when oseltamivir resistance develops and a person is unable to take zanamivir via the inhaled route [17]. The objective of this study is to develop and to validate the selected and sensitive LC-MS/MS method for the determination of peramivir in the plasma of rat and to gauge the pharmacokinetics of these compound after oral administration of exact samples in the rat. Fig. 1 shows the chemical structures of Peramivir and its related impurities.

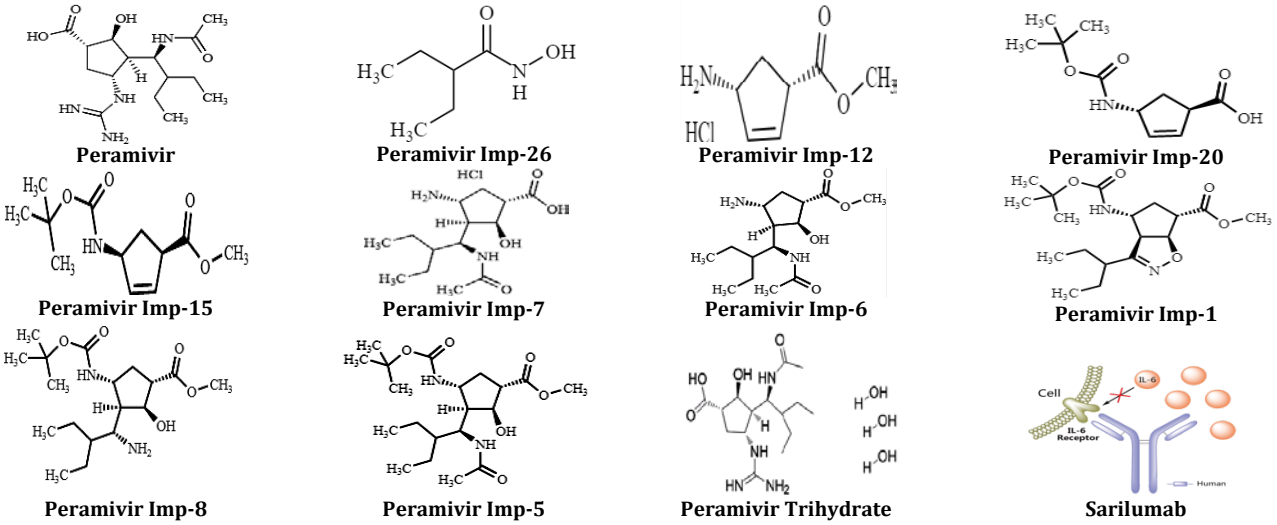

Fig. 1: Chemical structures of peramivir and its related impurities

In the present research, LC-MS was used for the simultaneous quantification of peramivir and its related impurities in rat plasma. Until now, there were no quantification methods for the estimation of peramivir and its related impurities. The present study was designed to investigate on (a) to create and approve a particular and delicate LC-MS/MS strategy regarding the assurance along with Peramivir and its related impurities plasma in rats, and (b) to assess the pharmacokinetics of these drugs after intravenous administration of test extracts in rats.

MATERIALS AND METHODS

Reagents (Chemicals and materials)

Reference standards for peramivir (99.9% purity) and its related impurities came from Cadila health care limited, Ahmedabad, India. HPLC marked acetonitrile; formic acid was obtained from Merck in Mumbai, India. HPLC grade Milli Q water is used for purification. (Milli Q system, USA).

Equipment

Waters alliance e-2695 model HPLC system was coupled to SCIEX QTRAP 5500 mass spectrometer with an electrospray ionization (ESI) interface [18, 19]. The SCIEX software [20-22] was used to interpret the chromatogram data. Column waters X-bridge C_{18} was used for separation and validation.

Conditions of the mass spectrometer

Multiple reaction monitoring (MRM) of the mass spectrometer with positive ion electrospray ionization mode (+ESI) was used for the separation of peramivir and its related substances. Collision energy of 15V and 14V, source temperature of 550 °C, ion spray voltage of 5500V, drying gas temperature of 120-250 °C, collision gas of nitrogen, inlet and outlet potential of 10V, 7V and Dwell time of 1 sec was used in mass spectrometer.

Conditions of chromatography

A mixture of 0.1% formic acid and acetonitrile was used as a mobile phase with gradient elution. 10 µl of injection volume and 1 ml/min of flow rate was used for this validation.

Standard solution preparation

By diluting with diluents, the standard solution of peramivir (50 ng/ml), imp-26 (10 ng/ml), imp-12 (5 ng/ml), imp-20 (10 ng/ml), imp-15 (10 ng/ml), imp-7 (10 ng/ml), imp-6 (5 ng/ml), imp-1 (20 ng/ml), imp-8 (15 ng/ml), peramivir trihydrate (active metabolite) (30 ng/ml), imp-5 (20 ng/ml), Sarilumab (internal standard) (50 ng/ml) were prepared. The standard solutions were stored at 4 °C and brought back to room temperature before use.

Table 1: **Gradient program**

Time (min)	Acetonitrile	Buffer (0.1% Formic acid)
0	20	80
10	50	50
20	70	30
30	20	80
40	20	80

Sample solution preparation

By adding 200 µl of plasma, 800 µl of acetonitrile, 500 µl of internal standard and 500 µl of standard stock, the sample solution was prepared. Mix in the vortex cyclomixture to precipitate all the proteins. Centrifuge for 20 min at 400 rpm, collect and inject the supernatant solution into the HPLC system.

Pharmacokinetic study

Selection of animals

In this study six healthy white albino rats (body weight between 250-350grams) were obtained from Biological E Limited, Hyderabad, India. The protocol of the animal study was approved by the institute of the animal ethics committee (Reg. No: 1074/PO/Re/S/05/CPCSEA). Six rats are under fasting condition. Blood samples were collected from cardiac puncture procedure. The rat is anesthetized and blood is collected via the left ventricle using a 19-21 gauge needle. Blood will be withdrawn slowly to prevent the heart from collapsing. vein with volume of 0.2 ml to 0.4 ml at 0, 0.3, 0.5, 0.75, 1, 1.5, 2, 4, 6, 8, 10 and 12 h. Each sample was separated by centrifugation and stored at-20 °C.

Method validation

The method was validated [23-31] in selective, sensitive, linearity, accuracy and precision, matrix condition, recovery study, re-injection reproducibility and stability.

Selectivity

The optimized LC-MS/MS method was determined by an analysis of 6 lots of individual rat plasma samples. Chromatograms of spiked rat plasma samples at the LLOQC level were compared with those of blank plasma samples.

Effect of the matrix

The matrix effect [32, 33] of rat plasma on the simultaneous analysis of peramivir was evaluated by comparing the peak area of peramivir in the extracted blank plasma with those of peramivir standard solution. It has been studied at three replicates of LQC and HQC levels.

Integrity of dilution

Integrity of dilution [34, 35] should be demonstrated by splitting the matrix with an analyte concentration above the ULOQC and diluting the sample with a blank matrix.

Accuracy and precision

Intraday precision and accuracy were tested in six replicates in a single set using samples of HQC, LQC, MQC and LLOQC concentrations. Inter-day precision and accuracy were tested by HQC, LQC, MQC and LLOQC in three separate batches. The accuracy was expressed as a percent CV and accuracy as percent recovery.

Carryover

Carryover [36, 37] is the small quantity of analyte present by the chromatographic system during the sample injection, which appears empty or unknown in subsequent samples.

Recovery

The extraction efficiency of peramivir was determined by an analysis of six replicates at each quality control concentration. The percentage recovery was assessed by comparing the peak areas of the extracted standards to the peak areas of the non-extracted standards.

Stability

Stability [38, 39] solutions were achieved by comparing the area response of the analyte in the stability sample with the area response of the sample prepared from the fresh stock solution. Plasma stability studies were conducted at HQC and LQC levels using six replicates at each level. The analyte was considered stable if the change is less than 15% as per USFDA guidelines. The stability of spiked rat plasma samples stored at room temperature (bench top stability) was evaluated for 24 h. The stability of the spiked rat plasma stored at 2-8 °C in the autosampler (autosampler stability) was evaluated for 24 h. The stability of the autosampler was evaluated by comparing the plasma extract samples that were immediately injected with the samples that were reinjected in the

auto sampler for 24 h at 2-8 °C. Frozen thaw stability was achieved by comparing the stability samples frozen at-30 °C and thawed three fold with freshly spiked internal control samples. Six aliquots of each of the concentrations of LQC and HQC were used for the stability assessment of freeze-thaw. In the long-term stability assessment, the concentration obtained after 24 h was compared with the initial concentration.

RESULTS AND DISCUSSION

Electro sapray ionization (ESI) with maximum response over atmospheric pressure chemical ionization (APCI) mode selected by this method. Optimization of the instrument to provide sensitivity and signal stability during the continuous flow of the mobile phase analyte into the electrospray ion source operated at a flow rate of 10 µl/min at both polarities. Peramivir gives more response in positive ion mode when compared with negative ion mode.

Various columns such as C_{18}, C_8 and CN-propyl and mobile phases consisting of 0.1 percent formic acid and acetonitrile were tested to obtain the best chromatographic condition. The best chromatographic condition occurred in the waters symmetry C_{18} with a mobile phase of 0.1 percent formic acid and acetonitrile in gradient elution with a flow rate of 1 ml/min.

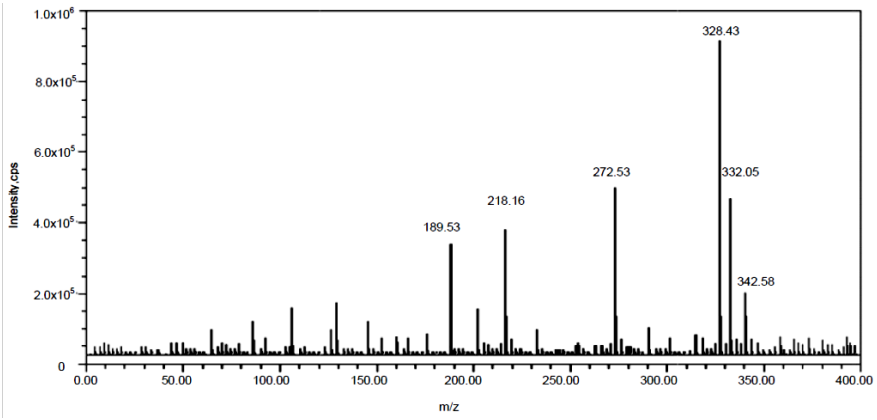

Fig. 2: Mass spectra of peramivir

Sensitivity

Blank plasma and spiked plasma with LOQ sample in of peramivir and its impurities. The percent interference of analyte retention time between six different batches of rat plasma, including hemolyzed and lipedemic plasma containing K_2EDTA as an anti-peramivir coagulant, is within the acceptable criteria. Six replicates of extracted samples were prepared and analyzed at LLOQC level in one of the plasma sample with the least interference at peramivir retention time. The percent CV of the area ratios of these six replicates of samples was found to be within the acceptable limit.

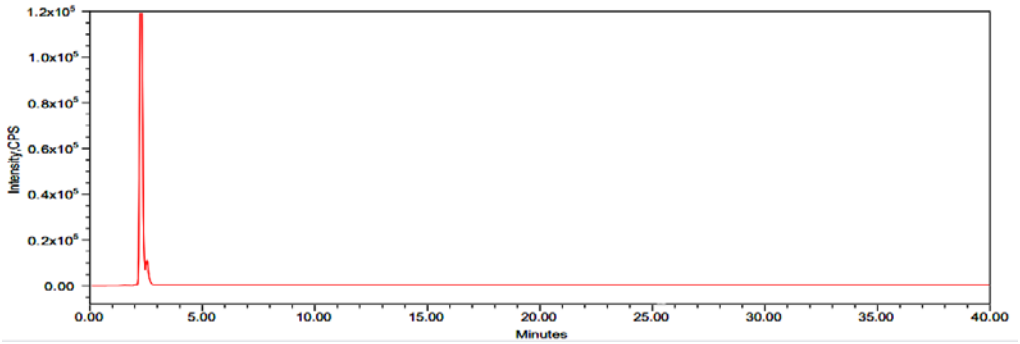

Fig. 3: Blank plasma chromatogram of peramivir

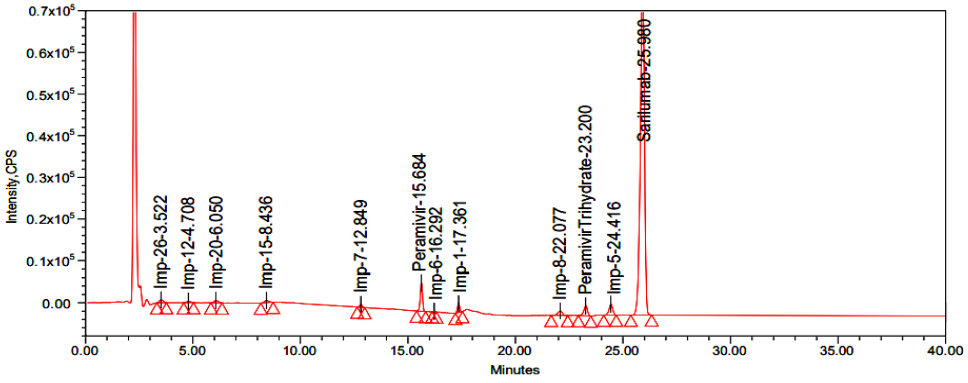

Fig. 4: LLOQC chromatogram of peramivir and its related substances

Table 2: A and B are the linearity results of peramivir and its impurities

Table A

Linearity	Per conc (ng/ml)	Per res	Imp26 Conc (ng/ml)	Imp26 res	Imp12 conc (ng/ml)	Imp 12 res	Imp20 conc (ng/ml)	Imp 20 res	Imp15 conc (ng/ml)	Imp 15 res
Linearity-1	5	0.1	1	0.01	1	0.005	1	0.012	1	0.010
Linearity-2	12.5	0.25	2.5	0.025	2.5	0.013	2.5	0.030	2.5	0.025
Linearity-3	25	0.5	5	0.05	5	0.025	5	0.060	5	0.050
Linearity-4	37.5	0.75	7.5	0.075	7.5	0.038	7.5	0.090	7.5	0.075
Linearity-5	50	1	10	0.1	10	0.05	10	0.120	10	0.100
Linearity-6	62	1.25	12.5	0.125	12.5	0.063	12.5	0.150	12.5	0.125
Linearity-7	75	1.5	15	0.15	15	0.071	15	0.180	15	0.150
Linearity-8	100	1.957	20	0.19	20	0.096	20	0.236	20	0.189
Slope	0.02		0.00968		0.0048		0.0119		0.0096	
Intercept	0.01		0.0015		0.0010		0.0006		0.0017	
CC	0.99987		0.99926		0.99926		0.99992		0.99910	

Table B

Linearity	Imp7 conc (ng/ml)	Imp 7 res	Imp6 conc (ng/ml)	Imp 6 res	Imp1 conc (ng/ml)	Imp 1 res	Imp8 conc (ng/ml)	Imp 8 res	Per hyd conc (ng/ml)	Per hyd res	Imp5 conc (ng/ml)	Imp 5 res
Linearity-1	1	0.012	0.5	0.002	2	0.035	1.5	0.020	3	0.050	2	0.035
Linearity-2	2.5	0.030	1.25	0.005	5	0.088	3.75	0.050	7.5	0.125	5	0.088
Linearity-3	5	0.060	2.50	0010	10	0.175	7.5	0.100	15	0.250	10	0.175
Linearity-4	7.5	0.090	3.75	0.015	15	0.263	11.25	0.150	22.5	0.375	15	0.265
Linearity-5	10	0.120	5	0.020	20	0.350	15	0.200	30	0.500	20	0.350
Linearity-6	12.5	0.150	6.25	0.025	25	0.438	18.75	0.250	37.5	0.625	25	0.438
Linearity-7	15	0.180	7.5	0.030	30	0.525	22.5	0.300	45	0.750	30	0.525
Linearity-8	20	0.232	10	0.038	40	0.661	30	0.377	60	0.948	40	0.662
Slope	0.0117		0.0039		0.0169		0.0128		0.0161		0.0169	
Intercept	0.0012		0.0003		0.0061		0.0035		0.0078		0.0062	
CC	0.99968		0.99926		0.99907		0.99902		0.99920		0.99909	

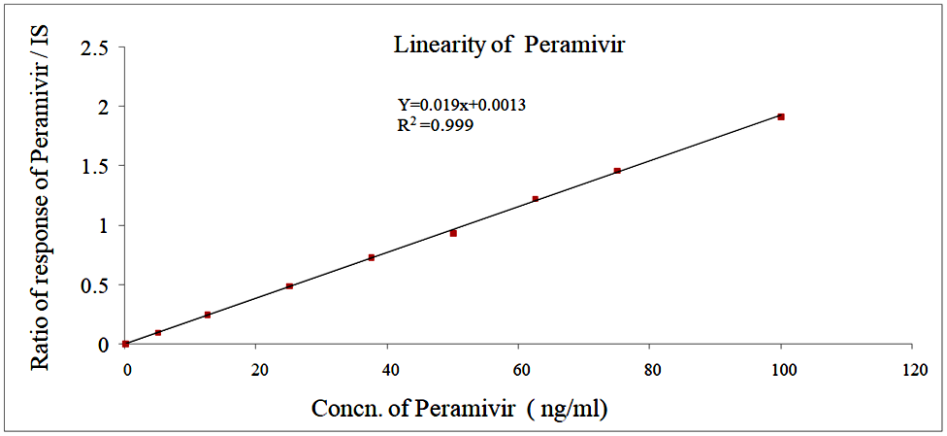

Fig. 5: Linearity plot of peramivir

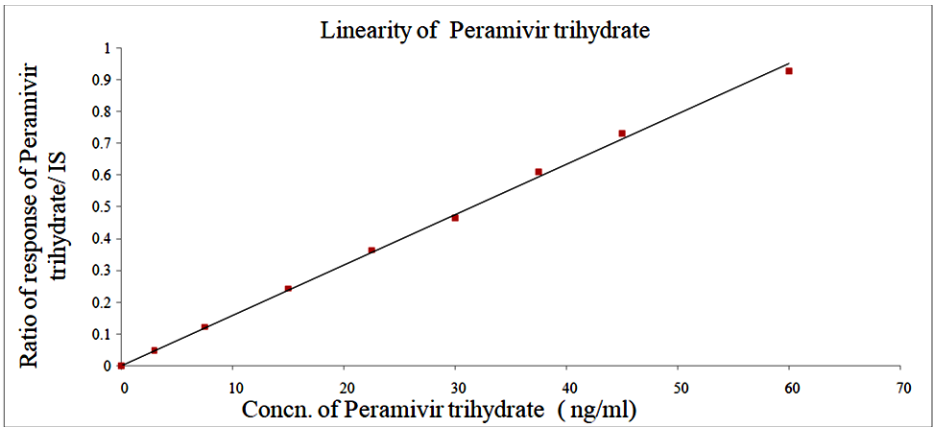

Fig. 6: Linearity plot of peramivir trihydrate

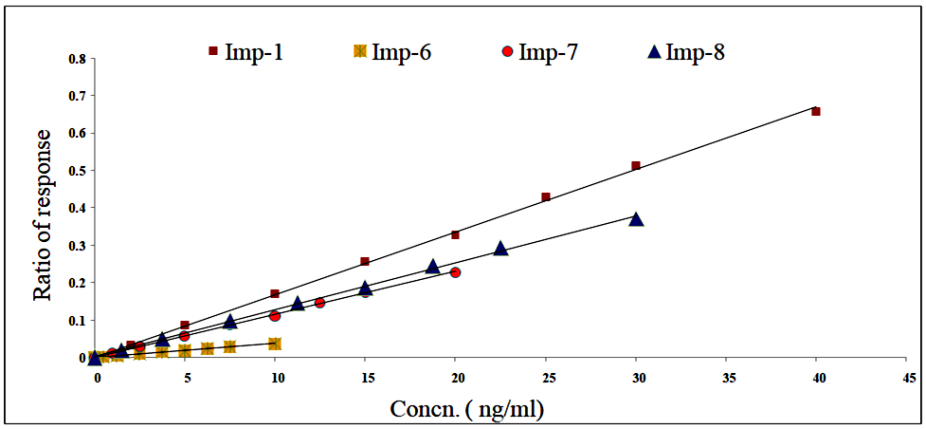

Fig. 7: Linearity plot of peramivir Imp-1, Imp-6, Imp-7 and Imp-8

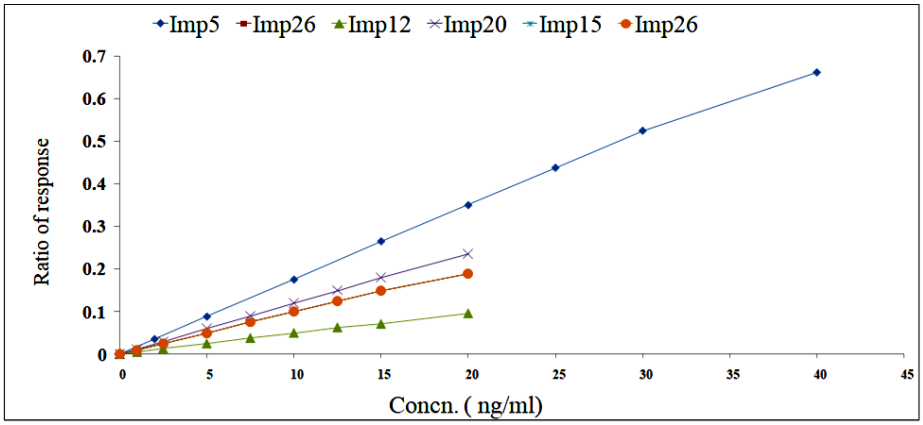

Fig. 8: Linearity plot of peramivir Imp-5, Imp-26, Imp-12, Imp-15, Imp-20 and Imp-26

Matrix effect

The ion suppression/enhancement percentage of CV in the signal was found to be 0.1 percent in MQC levels of Peramivir. It indicates that the effect of the matrix [40, 41] on the ionization of the analyte is within the acceptable limit.

Linearity

It was clear from the calibration curve that the peak area ratios were proportional to the concentration. The peramivir and its related compound solutions were prepared in the concentration range of 10% to 200%. The calibration curve was linear and the correlation coefficient was found to be 0.999. The linearity results of peramivir and its related compounds were shown in the following table [42].

Precision and accuracy

By pooling all individual assay results of different internal control samples, the accuracy and precision [43] were calculated. It was obvious, based on the data provided, that the strategy was precise and effective. The precision results of Peramivir and its related substances were shown in table 3.

Recovery

For recovery determination low, medium and high-quality control concentrations for peramivir and its related substances have been prepared and the areas collected for extracted samples of the same concentration levels from a precision and accuracy batch run on the same day. The mean recovery of peramivir was 100.13 and the precision was 1.2 percent.

Carryover

System error, which may affect the measured value of the sample, is called carryover. Based on the following procedure was evaluated through LC-MS/MS system, which was configured by waters alliance. System blank injection of 10 µl, 0.1 percent formic acid and acetonitrile in gradient mode into the water Z spray triple quadrupole mass detector was performed using a flow injection analysis. From this, we can say that it does not affect the accuracy and precision of the method proposed. Sample carryover is expressed as percent carryover.

Reinjection and reproducibility

During the actual sample analysis, reinjection reproducibility was performed to check the device after hardware deactivation due to any instrument failure. At LQC and HQC levels, the change was less than 2.0 and therefore, the batch was reinjected in the case of instrument failure during the actual subject sample analysis. Samples were prepared and reinjected after 24 h showing that the percent change was less than 2.0 percent at LQC and HQC levels and; therefore, the batch can be reinjected after 24 h during the actual sample analysis in the event of instrument failure.

Stability

Peramivir and its related substances solutions were prepared and stored in a refrigerator at 2-8 °C for solution stability analysis. Fresh stock solutions were developed 24 h earlier in relation to aged stock solutions. It is clear that the sample solutions were stable up to 24 h by observing the values of peramivir and its related substances.

Peramivir was stable in plasma for 24 h at room temperature and in an autosampler at 20 °C for 24 h. It has been confirmed that repeating freezing and thawing of plasma samples spiked with peramivir and its related substances did not affect their stability at LQC and HQC. Long-term stability showed that peramivir was stable at a storage temperature of-30 °C for up to 24 h. In the following table, the overall stability results of peramivir were tabulated.

Table 3: Precision and accuracy results of peramivir and its related substances

Name	Nominal conc (ng/ml)	Within run			Between run		
		Mean conc	Standard deviation	accuracy	Mean conc	Standard deviation	Accuracy
Peramivir	5	4.98	0.214	99.8	4.99	0.207	99.6
	25	25.01	0.748	100.1	25.02	0.726	100.2
	50	49.99	0.362	98.9	50.01	0.384	99.9
	75	75.02	0.159	100.2	74.98	0.147	98.9
Imp-26	1	0.99	0.854	98.7	1.01	0.868	99.7
	5	4.98	0.462	99.6	5.02	0.496	100.1
	10	10.02	0.153	99.9	9.98	0.151	98.6
	15	15.01	0.524	100.1	14.99	0.572	98.8
Imp-12	0.5	0.51	0.274	99.8	0.49	0.213	99.9
	2.5	2.52	0.163	100.1	2.51	0.108	100.1
	5	4.99	0.584	98.7	4.98	0.574	99.6
	7.5	7.51	0.721	99.9	6.99	0.698	98.7
Imp-20	1	0.99	0.639	98.6	1.02	0.619	100.2
	5	5.01	0.310	99.9	5.02	0.313	100.1
	10	9.98	0.527	98.8	9.99	0.557	99.8
	15	15.02	0.495	100.2	14.98	0.478	98.9
Imp-15	1	1.01	0.837	100.1	0.99	0.816	99.7
	5	4.98	0.754	98.5	5.01	0.743	100.1
	10	10.02	0.778	100.2	10.01	0.778	99.9
	15	14.99	0.637	99.7	15.03	0.613	100.2
Imp-7	1	0.98	0.485	99.4	0.99	0.441	99.5
	5	5.02	0.129	99.9	4.98	0.126	99.9
	10	9.99	0.384	98.9	10.01	0.396	100.1
	15	15.01	0.754	100.1	15.03	0.778	100.2
Imp-6	0.5	0.51	0.298	99.9	0.49	0.283	99.7
	2.5 l	2.52	0.854	100.1	2.48	0.821	98.8
	5	4.99	0.085	98.8	5.01	0.159	99.9
	7.5	7.48	0.074	98.5	7.51	0.084	100.1
Imp-1	2	1.96	0.845	99.4	1.98	0.831	99.6
	10	10.01	0.374	99.9	9.99	0.352	99.8
	20	20.02	0.473	100.1	20.02	0.496	100.1
	30	29.98	0.985	99.6	29.97	0.867	99.6
Imp-8	1.5	1.49	0.821	98.8	1.51	0.881	99.9
	7.5	7.51	0.364	100.1	7.48	0.352	98.7
	15	14.96	0.874	99.3	15.01	0.745	99.9
	22.5	22.53	0.855	100.3	22.49	0.766	98.5
Peramivir Trihydrate	3	2.98	0.827	99.6	3.01	0.859	100.1
	15	15.01	0.638	99.9	14.99	0.662	98.9
	30	29.99	0.096	98.7	30.01	0.145	100.1
	45	45.02	0.381	100.1	44.98	0.372	99.8
Imp-5	2	2.01	0.874	99.9	1.97	0.866	98.7
	10	9.99	0.772	98.9	10.02	0.735	100.2
	20	19.94	0.193	98.3	19.97	0.167	98.9
	30	30.1	0.589	100.1	29.98	0.553	99.8

mean±SD (n=6)

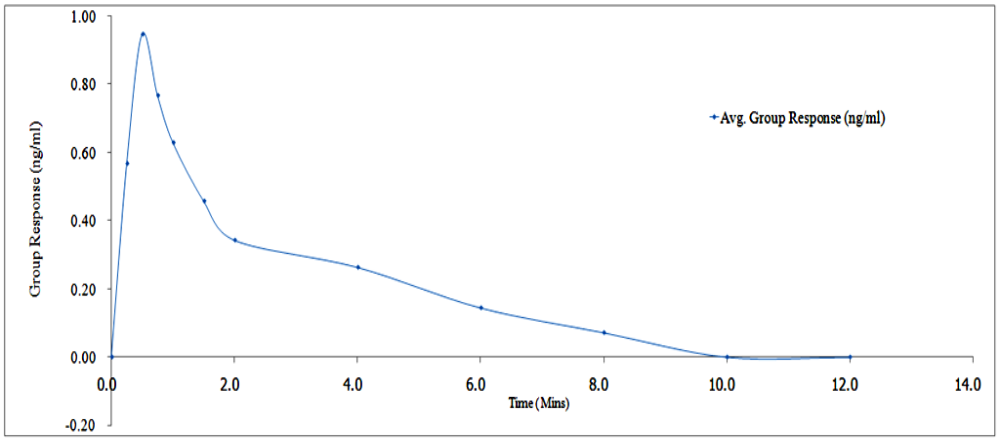

Fig. 6: Recovery plot of peramivir

Table 4: Stability results of peramivir and its impurities

Name	Conc level	Bench top stability	Auto sampler stability	Long term stability	Freeze thaw stability	Wet extract stability	Dry extract stability	Short term stability
		mean±SD						
Peramivir	LQC	25.31±0.525	25.17±0.341	25.22±0.415	25.27±0.341	25.16±0.258	25.43±0.138	25.53±0.621
	MQC	50.42±0.757	50.28±0.417	50.04±0.857	50.13±0.274	50.74±0.386	50.04±0.625	50.43±0.358
	HQC	75.02±0.162	75.16±0.532	75.16±0.234	75.41±0.136	75.58±0.451	75.31±0.417	75.15±0.557
Imp 26	LQC	5.10±0.326	5.23±0.534	5.12±0.741	5.14±0.534	5.07±0.412	5.23±0.536	5.34±0.254
	MQC	10.30±0.024	10.21±0.174	10.52±0.132	10.21±0.174	10.63±0.215	10.17±0.274	10.26±0.534
	HQC	15.42±0.174	15.74±0.235	15.62±0.085	15.14±0.235	15.38±0.745	15.06±0.552	15.29±0.284
Imp 12	LQC	2.51±0.721	2.55±0.724	2.66±0.441	2.63±0.775	2.54±0.637	2.55±0.652	2.59±0.632
	MQC	5.04±0.624	5.13±0.126	5.15±0.374	5.12±0.632	5.15±0.742	5.46±0.534	5.12±0.427
	HQC	7.53±0.531	7.62±0.385	7.58±0.475	7.59±0.312	7.38±0.629	7.53±0.847	7.55±0.534
Imp 20	LQC	5.24±0.325	5.32±0.274	5.41±0.325	5.07±0.263	5.26±0.342	5.42±0.314	5.63±0.157
	MQC	10.32±0.418	10.38±0.326	10.18±0.374	10.18±0.745	10.74±0.621	10.62±0.475	10.43±0.528
	HQC	15.17±0.625	15.65±0.296	15.26±0.124	15.37±0.218	15.51±0.295	15.32±0.527	15.55±0.641
Imp 15	LQC	5.23±0.185	5.12±0.462	5.57±0.342	5.22±0.203	5.74±0.635	5.15±0.210	5.53±0.241
	MQC	10.63±0.241	10.84±0.552	10.19±0.253	10.25±0.742	10.08±0.523	10.63±0.748	10.53±0.221
	HQC	15.53±0.628	15.37±0.436	15.41±0.743	15.34±0.625	15.42±0.736	15.25±0.784	15.34±0.163
Imp 7	LQC	5.74±0.154	5.38±0.745	5.14±0.248	5.42±0.107	5.27±0.324	5.84±0.241	5.54±0.315
	MQC	10.03±0.857	10.12±0.365	10.32±0.645	10.54±0.523	10.16±0.524	10.36±0.285	10.22±0.341
	HQC	15.62±0.558	15.24±0.625	15.85±0.341	15.09±0.274	15.19±0.325	15.09±0.713	15.26±0.437
Imp 6	LQC	2.57±0.587	2.55±0.216	2.58±0.421	2.55±0.324	2.51±0.074	2.54±0.421	2.52±0.369
	MQC	5.36±0.396	5.24±0.427	5.21±0.748	5.46±0.352	5.26±0.375	5.85±0.635	5.27±0.413
	HQC	7.59±0.234	7.56±0.527	7.53±0.129	7.59±0.743	7.55±0.262	7.53±0.182	7.58±0.134
Imp 1	LQC	10.32±0.745	10.24±0.745	10.34±0.754	10.17±0.325	10.85±0.574	10.18±0.742	10.54±0.274
	MQC	20.36±0.526	20.52±0.341	20.24±0.136	20.12±0.624	20.35±0.285	20.31±0.463	20.32±0.457
	HQC	30.62±0.475	30.74±0.659	30.58±0.298	30.74±0.853	30.34±0.625	30.52±0.964	30.16±0.522
Imp 8	LQC	7.52±0.328	7.53±0.528	7.56±0.417	7.59±0.742	7.48±0.638	7.58±0.324	7.49±0.375
	MQC	15.42±0.162	15.63±0.487	15.35±0.852	15.83±0.447	15.64±0.754	15.174±0.385	15.143±0.328
	HQC	22.56±0.638	22.53±0.558	22.55±0.743	22.53±0.164	22.49±0.327	22.52±0.463	22.62±0.748
Peramivir	LQC	15.32±0.745	15.27±0.321	15.64±0.425	15.64±0.354	15.47±0.328	15.23±0.524	15.65±0.632
Trihydrate	MQC	30.56±0.857	30.36±0.421	30.35±0.124	30.18±0.689	30.24±0.748	30.57±0.882	30.14±0.174
	HQC	45.23±0.857	45.54±0.856	45.18±0.746	45.74±0.362	45.06±0.819	45.34±0.642	45.36±0.842
Imp 5	LQC	10.24±0.748	10.15±0.749	10.32±0.685	10.74±0.698	10.34±0.457	10.39±0.547	10.63±0.742
	MQC	20.56±0.235	20.45±0.843	20.64±0.487	20.35±0.241	20.63±0.285	20.48±0.352	20.08±0.421
	HQC	30.41±0.624	30.55±0.624	30.35±0.648	30.49±0.374	30.17±0.241	30.49±0.748	30.39±0.052

mean±SD (n=6)

Table 5: Mean pharmacokinetic parameters of peramivir

Time (h)	Mean response for 6-rats
0.0	0.00
0.3	0.578
0.5	0.954
0.75	0.784
1.0	0.610
1.5	0.480
2.0	0.350
4.0	0.287
6.0	0.140
8.0	0.070
10.0	0.000
12.0	0.000
T_{max}	30 min
C_{max}	0.954
$T_{1/2}$	12H
$AUC_{(0-t)}$	4 ng-h/ml
$AUC_{(0-\infty)}$	4 ng-h/ml
$AUMC_{(0-t)}$	4 ng-h*h/ml
$AUMC_{(t-\infty)}$	315 ng-h*h/ml
$AUMC_{(0-\infty)}$	320-h*h/ml

Pharmacokinetic study

The liquid-liquid extraction method was used to isolate Peramivir in rat plasma. For this, 200 µl of plasma sample (respective concentration) were added into labelled polypropylene tubes and vortexed briefly; after that 300 µl of acetonitrile was added and vortexed for 10 min followed by centrifuged at 4000 rpm at 20 °C. After that, the separated aqueous layer was filtered with 0.45µ syringe filter.

Peramivir was administered as an oral dose under fasting condition of different groups of rats [44, 45]. After the drug samples are injected into the rat body [46, 47], the samples are collected at selected intervals of time, such as 30 min. After that, the samples were prepared as per the above procedure and injected into the chromatographic system and the values are recorded. The calculated accurate bioavailability of dosage of intravenous injection, C_{max} after intravenous administration of Peramivir (0.954), T_{max} (30 min), K_{el} (obvious first request terminal rate constant calculated from semi-log plot of plasma concentration versus time bend, using the least square relapse technique and $t_{1/2}$ (terminal half-life as governed by $0.693/K_{el}$ quotient). Test/reference ratio for $C_{max,}$ AUC_{0-t} and $AUC_{0-\infty}$ were 0.954, 4 ng-h/ml, 4 ng-h/ml, respectively, and found to be within the acceptable limit. Table 5 gives the Pharmacokinetic parameters [48, 49] of Peramivir.

CONCLUSION

The higher sensitive LC MS/MS method for the determination of peramivir in rat plasma has been developed and validated for the first time. In comparison to the protein precipitation method, we have developed liquid-liquid extraction for sample preparation with increased sensitivity as well as increased column life. The described method here is a robust, reproducible method of bioanalysis. Easy and systematic methods have been developed and can be used in pharmacokinetic studies and in the body fluids to check the analyte being examined.

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AUTHORS CONTRIBUTIONS

All authors have contributed equally.

CONFLICTS OF INTERESTS

The authors are conformed no conflicts of interest.

REFERENCES

1. Hayden FG, de Jong MD. Emerging influenza antiviral resistance threats. J Infect Dis. 2011;203(1):6-10. doi: 10.1093/infdis/jiq012, PMID 21148489.
2. Lindegren ML, Griffin MR, Williams JV, Edwards KM, Zhu Y, Mitchel E. Antiviral treatment among older adults hospitalized with influenza, 2006-2012. PLOS ONE. 2015;10(3):e0121952. doi: 10.1371/journal.pone.0121952, PMID 25807314.
3. Saunders-Hastings PR, Krewski D. Reviewing the history of pandemic influenza: understanding patterns of emergence and transmission. Pathogens. 2016;5(4):66. doi: 10.3390/pathogens5040066, PMID 27929449.
4. Dabestani NM, Leidner AJ, Seiber EE, Kim H, Graitcer SB, Foppa IM. A review of the cost-effectiveness of adult influenza vaccination and other preventive services. Prev Med. 2019;126:105734. doi: 10.1016/j.ypmed.2019.05.022, PMID 31152830.
5. Gubareva LV. Molecular mechanisms of influenza virus resistance to neuraminidase inhibitors. Virus Res. 2004;103(1-2):199-203. doi: 10.1016/j.virusres.2004.02.034, PMID 15163510.
6. Sugaya N. Widespread use of neuraminidase inhibitors in Japan. J Infect Chemother. 2011;17(5):595-601. doi: 10.1007/s10156-011-0288-0, PMID 21850418.
7. Gutierrez JA, Luo M, Singh V, Li L, Brown RL, Norris GE. Picomolar inhibitors as transition-state probes of 5′-methylthioadenosine nucleosidases. ACS Chem Biol. 2007;2(11):725-34. doi: 10.1021/cb700166z, PMID 18030989.
8. Schramm VL. Enzymatic transition states, transition-state analogs, dynamics, thermodynamics, and lifetimes. Annu Rev Biochem. 2011;80:703-32. doi: 10.1146/annurev-biochem-061809-100742, PMID 21675920.
9. Yin J, Redovich J. Kinetic modeling of virus growth in cells. Microbiol Mol Biol Rev. 2018;82(2). doi: 10.1128/MMBR.00066-17, PMID 29592895.
10. Blaas D. Viral entry pathways: the example of common cold viruses. Wien Med Wochenschr. 2016;166(7-8):211-26. doi: 10.1007/s10354-016-0461-2, PMID 27174165.
11. Fujii T, Udy A, Licari E, Romero L, Bellomo R. Sodium bicarbonate therapy for critically ill patients with metabolic acidosis: a scoping and a systematic review. J Crit Care. 2019;51:184-91. doi: 10.1016/j.jcrc.2019.02.027, PMID 30852347.
12. Lv L, Zhang J. The incidence and risk of infusion phlebitis with peripheral intravenous catheters: A meta-analysis. J Vasc Access. 2020;21(3):342-9. doi: 10.1177/1129729819877323, PMID 31547791.
13. Griffith RJ, Jordan V, Herd D, Reed PW, Dalziel SR. Vapocoolants (cold spray) for pain treatment during intravenous cannulation. Cochrane Database Syst Rev. 2016;4:CD009484. doi: 10.1002/14651858.CD009484.pub2, PMID 27113639.
14. Nicoll LH, Hesby A. Intramuscular injection: an integrative research review and guideline for evidence-based practice. Appl Nurs Res. 2002;15(3):149-62. doi: 10.1053/apnr.2002.34142, PMID 12173166.
15. Cook IF. Best vaccination practice and medically attended injection site events following deltoid intramuscular injection. Hum Vaccin Immunother. 2015;11(5):1184-91. doi: 10.1080/21645515.2015.1017694, PMID 25868476.
16. Richert L. Reagan, regulation, and the FDA: the US Food and Drug Administration's response to HIV/AIDS, 1980-90. Can J Hist. 2009;44(3):467-88. doi: 10.3138/cjh.44.3.467.
17. Passali D, Lauriello M, Bellussi L, Passali GC, Passali FM, Gregori D. Foreign body inhalation in children: an update. Acta Otorhinolaryngol Ital. 2010;30(1):27-32. PMID 20559470.
18. Potturi Ramadevi, Kantipudi Rambabu. Bioanalytical method development and validation for ezetimibe and pitavastain and its applications to pharmacokinetic studies in rabbit plasma by using LCMS/MS. IJRPS 2021;11(4):7854-62. doi: 10.26452/ijrps.v11i4.4670.
19. Eluru A, Surendra Babu K. Bioanalytical method development and validation for Aplidine in rat plasma and their pharmacokinetic studies by LCMS. WJPPS. 2019;8:1201-9.
20. Ramchandran D, Kethipalli A, Krishnamurthy M. Bioanalytical method development and validation of daunorubicin and cytrarabine in rat plasma by LC-MS/MS and its application in pharmacokinetic studies. J Pharm Sci Res. 2020;12:381-6.
21. Naykode MD, Bhagwat DA, Jadhav SD, More HN. Analytical and bioanalytical method for quantification of pure azilsartan, not its salts by RP-HPLC. Res J Pharm Technol. 2017;10(3):708-14. doi: 10.5958/0974-360X.2017.00133.0.
22. 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.
23. 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.
24. Senthil Rajan D, Muruganathan G, Shivkumar K, Ganesh T. Development and validation of HPLC method for simultaneous quantification of vasicine, glycyrrhizin and piperine in polyherbal cough syrup. Int J Curr Pharm Res. 2020;12:15-9.
25. 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.
26. 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. Crit Rev. 2020;7:4785-90.
27. kumar AS, Manidipa Debnath, Seshagiri Rao JVLN, Gowri Sankar D. 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. Research J Pharm and Tech 2016;9:549-54.
28. Malak Y, Al-Bathish AA, Gazy MK, El-Jamal. Rp-HPLC and chemometric methods for the determination of two antidiabetic mixtures; metformin hydrochloride-canagliflozin and metformin hydrochloride-gliclazide in their pharmaceutical formulation. Int J Pharm Pharm Sci. 2020;12:83-94.
29. 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.
30. Koya Prabhakara Rao NL, A Amara Babu, Kalyani Koganti, Babji Palakeeti, Koduri SV Srinivas. Relat subst method dev validation LC-Ms/MS method for the quantification of selexipag and its related impurities in rat plasma and its application to pharmacokinetic studies. SN Applied Sciences. 2021;3:321.
31. Hasanah YIF, 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.
32. Naveen VMK, Veeraswami B, Srinivasa Rao G. High response bioanalytical validation approach of nadolol and bendroflumethiazide by LC-MS/MS on rat plasma. Int J Res Pharm Sci. 2020;11:2272-9.
33. Kumari GK, Kantipudi R. Bioanalytical method development and validation for avapritinib in rat plasma by LC-MS/MS. J Pharm Sci Res. 2021;13:134-7.
34. 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.
35. Rao KP, babu NL, Koganti K, Palakeeti B, Srinivas KSV. 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(3):321. doi: 10.1007/s42452-021-04219-x.

36. Koya Prabhakara Rao, Namburi NL, A Amara Babu AA, Kalyani Koganti K, Babji Palakeeti B, Koduri SV, Srinivas KSV. Development and validation of UPLC method for separation and determination of rivaroxaban and its related substances in bulk drugs. Drug Invention Today. 2020;13:611-8.
37. Charu Pandya P, Sadhana Rajput J. Development and validation of stability indicating method RP-HPLC method of acotiamide. Int J Pharm Pharm Sci. 2018;10:1-8.
38. Athavia BA, Dedania ZR, Dedania RR, Swamy SMV, Prajapati CB. Stability indicating HPLC method for determination of vilazodone hydrochloride. Int J Curr Pharm Sci 2017;9(4). doi: 10.22159/ijcpr.2017v9i4.20975.
39. 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. Research J Pharm and Technol. 2013;6:200-3.
40. Swati K, Abhishek P, Sushank S, Bothiraja C, Atmaram P. High-performance liquid chromatography for the simultaneous estimation of cefoperazone and sulbactam in rat plasma and its importance in therapeutic drug monitoring. Int J Pharm Pharm Sci. 2020;12:92-7.
41. Vijayakumari M, Reddy Ch B. Stability indicating validated hplc method for the determination of zanubrutinib in bulk and pharmaceutical dosage form. Asian J Pharm Clin Res. 2020;13:159-62.
42. Raziq A. Syed Umer Jan. Relative comparison of stability and degradation of methylcobalamin tablets of different brands at different storage settings. Int J Appl Pharm. 2021;13:171-5.
43. Siva Madhu Chaitanya, Srinath Nissankararao, Satya Lakshmi Gandham. A sort of validated bioanalytical 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 bioequivalence studies. IJRPS 2021;12(1):708-17. doi: 10.26452/ijrps.v12i1.4167.
44. Prasanthi S, Himabindu G. Bıo anlaytıcal method for sımultaneous estımatıon of rıbocıclıb and letrozole and ıts applıcatıon to pharmacokınetıc studıes usıng ultra performance lıquıd chromatography. Int J Appl Pharm. 2022;14:95-102.
45. Syed R, Kantipudi R. Bıo-analytıcal method development and valıdatıon of avelumab, axıtınıb and ıts applıcatıon to pharmacokınetıc studıes ın rabbıt plasma by usıng LCMS/MS. Int J Appl Pharm. 2021;13:198-204.
46. Subba Rao Yarlagadda SR, Pavani Y, Subba Rao Mannam SR. Simultaneous method development and validation of trastuzumab and hyaluronidase-Oysk and its pharmacokinetic studies with LC-MS/MS. J Pharm Sci Res. 2020;12:375-80.
47. Subrahmanyam Talari S, Anuradha Vejendla A, Ratna Kumari Shetty R Kumari. Development and validation of a UPLC-MS/MS method for the simultaneous determination of verapamil and trandolapril in rat plasma: application to a pharmacokinetic study. Current Pharmaceutical Analysis. 2022;18(3):291-304. doi: 10.2174/1573412917666210302145711.
48. Prasanthi S, Himabindu G. Bioanalytical method for simultaneous estimation of ezetimibe and pitavastatin and its application to pharmacokinetic studies using uplc. YMERmer Journal. 2022;21(2):718-32. doi: 10.37896/YMER21.02/67.
49. Prasanthi S, Himabindu G. Assay of the bioanalytical method for the estimation of avelumab and aaxitinib using UPLC and its application to pharmacokinetic studies. Journal of Pharmaceutical Research International. 2022;34:1-10. doi: 10.9734/jpri/2022/v34i27A35988.