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Review Article

Quantification of Rivaroxaben and its related impurities in rat plasma using an LC-MS/MS and validated for pharmacokinetic studies, as well as its application

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ABSTRACT

In the treatment of DVT and pulmonary embolism (blood clots usually in the legs), Rivaroxaban is used (PE, blood clot in the lung). LC-MS/MS was used to separate rivaroxaban from any impurities. As a result of validation of the bio-analytical method and pharmacokinetic analysis of Rivaroxaban and its associated impurities in rat plasma, the present application seeks to be published. Impurity spiked solution was used to optimise the chromatographic technique. Rivaroxaban gradient elution with a flow rate of 1 ml/s and an inertsil ODS column (250mmx4.6mm, 5) make up the optimised system. As a mobile step, a solution of 1 ml formic acid in 1 lt water and acetonitrile is used. Rivaroxaban and its associated impurities, such as impurity-17, were isolated from Dabigatran as an internal standard and as an active metabolite over the course of a 40-minute run. The linearity curves were found to be linear between 10% and 200% of rat plasma, with an R^2 value of 0.999 for each analyte. In this case, precision, accuracy, recovery, and stability have all been met within the guidelines set by the US Food and Drug Administration. Researchers can use plasma from rats to look into pharmacokinetic studies using this method.

1. Introduction

When used in conjunction with other anticoagulants [1, 2] like Xarelto, the medicine Rivaroxaban can help treat or prevent blood clots from forming. Deep vein thrombosis [3] and pulmonary embolism [4] are treated with it, and it's also used to prevent blood clots in arrhythmia [5, 6] and during hip or knee surgery. It's swallowed whole.

People often complain about bleeding [7], which is a common side effect. Spinal hematoma [8] and anaphylaxis [9, 10] are both serious side effects that can occur with this medication. Whether it's safe to use while pregnant or breast-feeding is an open question [11]. There are fewer interactions with other substances when compared to wayfaring. Xa protein clotting factor is blocked, so it works.

In the extreme, bleeding can occur both externally and internally. When compared to warfarin, rivaroxaban causes less severe and fatal bleeding, but it also causes more stomach and intestinal bleeding [12, 13]. However, even though there is now an antidote for rivaroxan, the safety and efficacy of it are not as well known as the antidotes for older anticoagulants like warfarin (Vitamin K and prothrombin complex concentrate), which means that severe bleeding might be more difficult to control. [10].

2. Materials and Methods

Valacyclovir HCl ***Chemicals and reagents***

Reference requirements for rivaroxaban (99.9% purity) and its associated impurities (99.9% purity) Candila Health Care Ltd. in Ahmedabad, India provided the Reference requirements for rivaroxaban (99.9% purity) and its associated impurities (99.9% purity). Merck (India), Worli, Mumbai, India provided HPLC-labeled acetonitrile and formic acid. The Milli Q method (Milli Q method, USA) purified water was used to make HPLC grade water. Bharat Biotech Ltd in Hyderabad provided the rat plasma used in this study.

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Instrumentation

The HPLC device was coupled to the SCIEX QTRAP 5500 mass spectrometer fitted with an electrospray ionisation interface with the conditions of the splitter being positioned in front of the ESI source, allowing only 35 percent of the eluent to be entered. Standard operating source conditions for MS scanning rivaroxaban in positive ESI mode were optimised as follows, fragmentor voltage was set at 80V, capillary at 3000V, skimmer at 60V, nitrogen was used as a drying and nebulizing gas (45psi). Highly filtered nitrogen gas has been used as a collision gas.

Sample collection and preparation, as well as quality assurance sampling

The regular solution of rivaroxaban (20 ng/ml), imp-15 (4 ng/ml), imp-E (2 ng/ml), imp-28 (4 ng/ml), imp-B (4 ng/ml), imp-16 (4 ng/ml), imp-23 (2 ng/ml), imp-27 (8 ng/ml), imp-19 (6 ng/ml), imp-17 (active metabolite) (12 ng/ml), imp-26 (8 ng/ml), dabigatran (internal standard) (20 ng/ml) was developed by dilution with diluents. Prepared standard solutions have been processed at at 4°C and brought back to room temperature before use.

Planning a sample solution

Two hundred microliters of plasma were combined with three hundred microliters of acetonitrile, along with diluents and internal standards and standard stock in order to create the sample's solution. Precipitate both proteins with a vortex cyclomixture. 20 minutes at 400 rpm in a centrifuge. Inject the chromatographic system with the supernatant solution collected in a vial.

Table 1. *In vitro* drug release profile of Valacyclovir HCl floating tablets containing HPMC K15 M (F1-F3) and HMC K100M (F4-F6).

Time (hrs)	Cumulative % drug release					
	F1	F2	F3	F4	F5	F6
0	0	0	0	0	0	0
1	27.2±0.72	30±0.4	29±0.35	20.2±0.72	23.2±0.64	26±0.52
2	30.1±0.32	34±0.6	31.9±0.5	27.4±0.79	29.1±0.81	37±0.81
3	35.1±0.45	38.2±0.58	36±0.45	35.1±0.77	36±0.83	44±0.2
4	39.9±0.3	44±0.45	40.9±0.3	38.7±0.3	40±0.62	55.2±0.3
5	46±0.5	52.1±0.41	48±0.45	47.1±0.79	48.9±0.5	61±0.2
6	51±0.3	58±0.5	53±0.5	55±0.86	56.1±0.75	68±0.41
7	59±0.5	66±0.3	63.2±0.72	62±0.83	63.1±0.76	76.6±0.25
8	68.1±0.36	74±0.25	70.3±0.81	66.9±0.98	68.9±0.6	88±0.2
9	74±0.65	77.9±0.55	76.2±0.64	70.9±0.92	73.9±0.62	90±0.37
10	80.2±0.58	85±0.6	83±0.45	82±0.91	83±0.51	94±0.6
11	88.9±0.5	93.4±0.4	93±0.45	88.7±0.73	90.9±0.51	96±0.26
12	93.4±0.41	97.2±0.3	95.4±0.35	93.3±0.25	95.3±0.4	98.6±0.5

Table 2. *In vitro* drug release profile of Valacyclovir HCl floating tablets containing Xanthan gum (F7-F9) and Sodium Alginate (F10-F12).

Time (hrs)	Cumulative % drug release					
	F7	F8	F9	F10	F11	F12
0	0	0	0	0	0	0
1	18±0.3	19.8±0.62	16.1±0.56	24.6±0.52	26.1±0.76	20.8±0.72
2	25.8±0.7	28.9±0.65	24.2±0.49	28.9±0.79	29.4±0.78	24.5±0.5
3	31±0.6	33.1±0.66	28.9±0.45	38.5±0.55	39.9±0.65	28.8±0.8
4	39±0.61	40±0.7	35.9±0.5	47.2±0.31	49.1±0.51	39.1±0.98
5	46±0.66	48.9±0.36	42.1±0.41	50.8±0.83	53.2±0.85	47.9±0.9
6	50.1±0.85	54.1±0.51	48±0.8	58.5±0.41	59±0.53	50.9±0.95
7	58.2±0.92	62±0.6	56±0.7	62.5±0.52	63±0.71	54.5±0.51
8	60.1±0.56	67.8±0.72	59±0.3	69.6±0.52	70±0.95	60.8±0.72
9	65.9±0.85	72.1±0.32	63.9±0.65	78.8±0.91	81.1±0.45	74.5±0.51
10	70.1±0.95	80.8±0.41	68±0.8	86±0.96	87.9±0.55	82.9±0.85
11	78.1±0.7	90±0.45	79.9±0.5	90.5±0.66	92.2±0.72	89.9±0.9
12	85.9±0.8	92.1±0.55	84±0.5			

Method Validation

Selectivity

Six batches of individual plasma samples were analysed to see how well the LC-MS/MS technique optimised the results. The chromatograms of spiked plasma samples at LLQC could be distinguished from the chromatograms of the blank plasma samples previously mentioned.

Matrix effect

The rivaroxaban matrix effect was evaluated by comparing the peak area of the extracted plasma sample from six different rats, along with its impurities and IS. Three replicates of each test have been performed at both low and high quality levels.

Dilution integrity

Doping the matrix with a material accumulation greater than the ULOQC and mixing the selected sample with a blank matrix should be used to demonstrate dilution integrity.

Precision and accuracy

Six replicates of a single collection were used to test the precision and accuracy on the same day using samples from the LLOQC, LQC, MQC, and HQC levels. Three different batches of LLOQC, LQC, MQC, and HQC concentration samples were tested for interday precision and accuracy. As a result, the precision was calculated as a percentage of CV, while the accuracy was calculated as a percentage of recover.

Carry over

That which is detected in one sample but is absent from subsequent ones because it was retained by the chromatographic system during the injection of that sample.

Recovery

Six duplicates at each quality control concentration were analysed to find out how well rivaroxaban and its impurities withdrew from the body. The peak response of the non-extracted standards was used to calculate the recovery percentage.

Stability

In the stability study, stability is achieved with a typical area response prepared from a new solution by an area response and an internal norm. Six preparations were used in the stability tests, which were conducted at two accumulation levels: low and high. According to USFDA standards, samples must be stable at or below a concentration of 15%. For up to 24 hours, benchtop stability samples were stored in an auto sampler at 2-8°C. -30°C samples of freeze-thaw stability are frozen and thawed three times in comparison to a newly prepared control sample before being analysed. Each sample is tested for freeze-thaw stability using six different levels of LQ and HQ Q quality control. For 24 hours, wet extract stability samples must be kept below 10°C.

3. Results and Discussion

Analyte fusion was carried out with an instrument that provided sensitivity and signal stability at 10 µl/min at both poles during continuous movable phase flow to the electro spray ion source. Comparing the positive and negative ion modes of action, rivaroxaban and its impurities have a greater response when administered in the former mode.

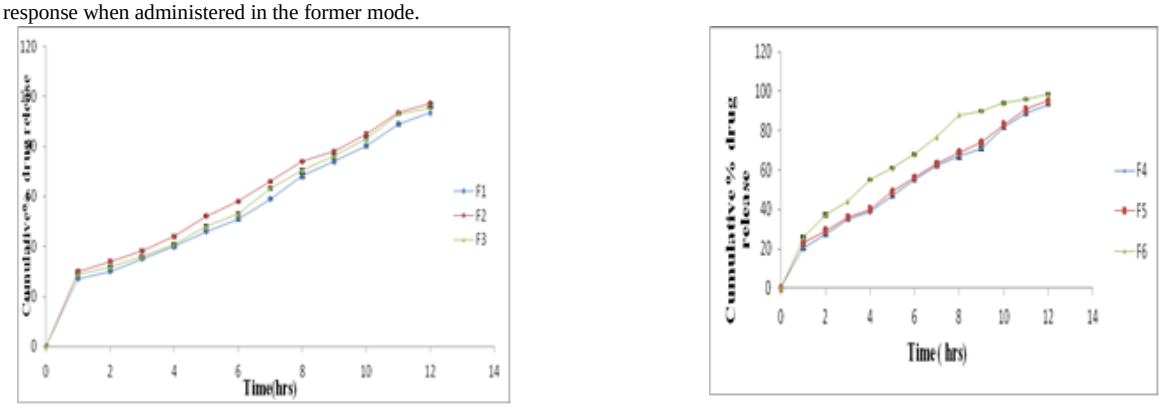

Figure 1. Dissolution Profile of Valacyclovir HCl floating tablets containing HPMC K15 M (F1, F2, F3) and HPMC K100 M (F4, F5, F6).

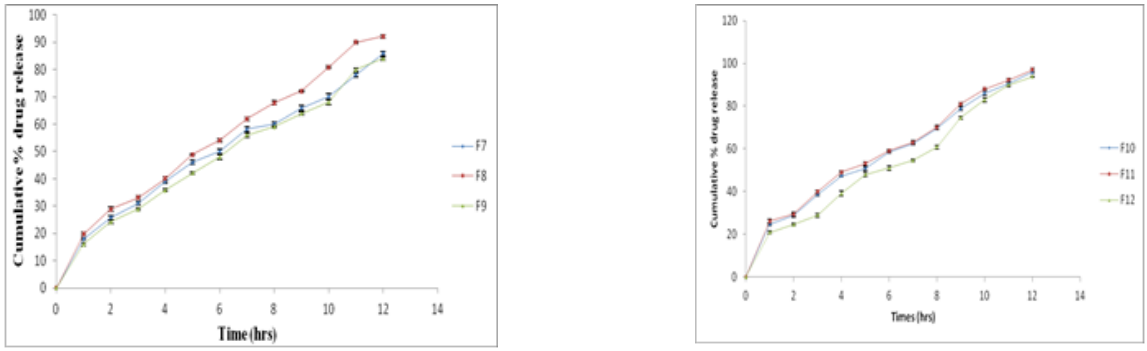

Figure 2. Dissolution Profile of Valacyclovir HCl floating tablets containing Xanthan gum (F7, F8, F9) and Sodium alginate (F10, F11, F12).

To find the simplest chromatographic state, a variety of columns, such as C18, C8, and CN-propyl, were tested along with mobile phases made up of 0.1 percent formic acid and acetonitrile. On the inertsil ODS column, using acetonitrile as the mobile phase and 0.1 percent formic acid as the elution rate gradient, the easiest chromatographic separation was achieved.

Validation process

Selectivity and Sensitivity

Spiked plasma and plasma containing a LOQ sample of the drug and its impurities, respectively. As an anti-coagulant, K2EDTA contains K2EDTA as an anti-coagulant for rivaroxaban. Therefore, the percent intervention in the retention period is within acceptable limits. Three plasma samples with minimal interference with rivaroxaban retention time were used to prepare six replicates of the extracted samples at LLOQC level. In these six replicates of samples, the rivaroxaban CV was 1.54 percent.

Mtrix effect

At the MQC stage, the ion suppression/enhancement percentage of CV for rivaroxaban and its impurities was found to be 1.0 percent. The results show that the matrix's influence on analyte ionisation is within the allowable range of effects.

Linearity

The calibration curve made it clear that the peak response ratios were inversely proportional to concentration. Rivaloxaban's regression coefficient was less than a thousandth of a percent.

Precision and accuracy

In order to find the inter-run and accuracy differences, all five individual batch runs examined on four different days were combined with the results of reproducing quality control. CV of inter-run precision was less than 5%, and inter-run accuracy ranged from 85 to 115% for both rivaroxaban and its impurities.

Recovery

Areas for extracted samples of the same accumulation amounts were prepared and collected from a precision and accuracy batch run on the same day for the recovery determination of rivaroxaban and its impurities LQC, MQC and HQC concentrations.

Carry over

Carry over refers to a device error that may have an impact on the sample's calculated value. We used this technique to examine samples that had been transferred to the LC-MS/MS system that was part of the Waters Alliance. A flow injection analysis was used to perform a machine blank injection of

10 µl 0.1 percent formic acid and acetonitrile in gradient mode in the Zspray triple quadrupole mass detector's water. As a result, the proposed method's accuracy and precision are unaffected. As well as a percent carryover, there was also an implied nl carryover in the sample data.

Re-injection and Re-Producibility

Samples were injected and reproduced to verify the instrument after hardware deactivation because of any instrument malfunction occurred during actual sample testing. As a result, the batch was re-injected in the event of an instrument failure during the actual subject test because the improvements at the LQC and HQC stages were less than 2.0. After 24 hours, the samples were prepared and re-injected, demonstrating that the percent change at LQC and HQC levels should be less than 2.0. As a result, in the event of an instrument failure, samples can be re-injected within 24 hours.

Stability

Rivaroxaban and its impurity solutions were prepared with diluents and stored at 2-8°C in a refrigerator for a solution stability analysis. Stock solutions prepared earlier than 24 hours were linked to older stock solutions. Stock solutions of rivaroxaban are stable for up to 24 hours when stored at 2-8°C, according to the percentage change values for the drug and its impurities. There was stability on the bench top as well as in the auto sampler during the LQC, MQC, and HQC stages.

At 20°C, the rivaroxaban and its impurities were stable in the auto sampler for 24 hours and in plasma for 24 hours when kept at room temperature. Therefore it was verified that plasma samples spiked with LQC and HQC levels of rivoxaban did not lose their stability after repeated freezing and thawing. The long-term stability of rivaroxaban and its impurities at -30°C for 24 hours was evident.

Pharmacokinetic studies

During fasting, rivaroxaban and its impurities were given orally to various groups of rats. Samples should be taken at time intervals of 0.5, 1, 2, 4, 6, 8, 10, 12, 14 and 16 hours after injection into the rat's body. The sample is then prepared according to the test procedure, injected into the chromatographic system, and the results are recorded. The pharmacokinetic parameters tested were C_{max}, t_{max} and $t_{1/2}$.

4. Conclusion

Rivaroxaban and its impurities have been detected for the first time using the more sensitive LC-MS/MS method developed and validated in rat plasma. The aforementioned technique is a bioanalytical method that is quick, reliable, and reproducible. Analyte regulation in body fluids and pharmacokinetic studies can both benefit from this simple and systematic

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Conflict of Interest

Authors announce that there have been no conflicts of interest.

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