STABILITY INDICATING ASSAY METHOD FOR THE QUANTIFICATION OF ASSAY OF (3S,4R)-3- ETHYL-4-{1,5,7,10-TETRA AZATRICYCLO [7.3.0.0 {2,6}] DODECA-2(6) ,3,7,9,11-PENTAEN-12-YL}- N-(2,2,2-TRIFLOURO ETHYL) PYRROLIDINE -1-CARBOXAMIDE TABLETS BY RP- HPLC.

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ABSTRACT:

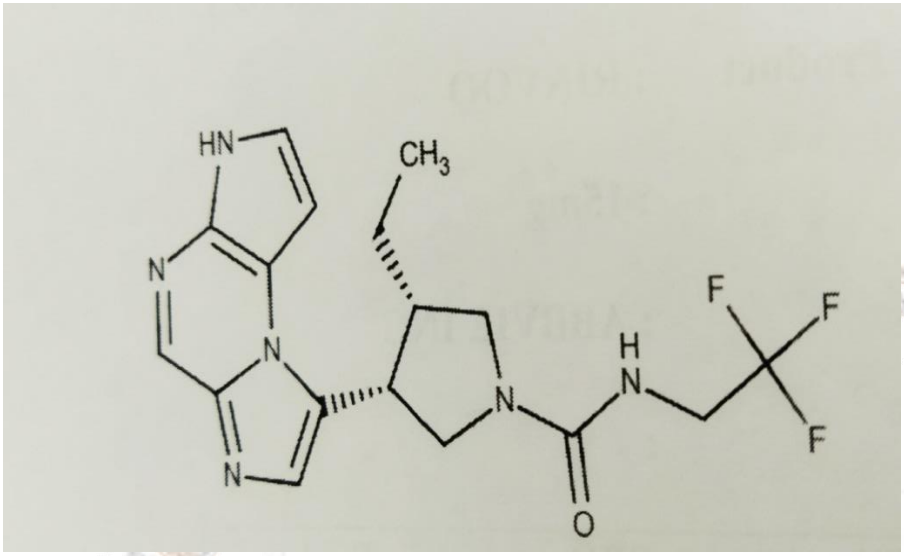
A rapid Reverse phase – High performance liquid chromatography was established for quantification of single drug, Upadacitinib hemihydrate. This method is simple, specific and stable. The estimation was done by using Inert sustain C18 (150×4.6) mm,5µ column Isocratic pump mode with flow rate-1.0 ml/min by using different concentrations from 25%-150% with correlation coefficient is less than 1.0.The Rt for upadacitinib was 10 mins. By using various chromatographic conditions like method validation, method development, specificity, system precision , robustness , accuracy the method is validated . The method was established and validated as per ICH guidelines.

KEY WORDS: Upadacitinib hemihydrate, potassium dihydrogen phosphate, sodium hydroxide, methanol, acetonitrile, RP-HPLC, UV , validation .

INTRODUCTION:

Upadacitinib, sold under brand name Rinvoq , is a janus kinase (JAK) inhibitor medication for the treatment of moderately to severely active rheumatoid arthritis and psoriatic arthritis in adults . It was approved for medical use in the united states and in the European union in 2019 and was developed by the biotech company Abbvic.on January 14,2022 Rinvoq received FDA approval for treatment refractory atopic dermatitis.

STRUCTURE:



TRADENAME-Rinvoq

IUPAC NAME: (3S,4R) – Ethyl-4-{1,5,7,10-tetraaza tricyclo[7.3.0.0{2,6}]dodeca-2(6),3,7,9,11-pentaen-12-yl}-N-(2,2,2-trifluoro ethyl pyrrolidine-1- caboxamide.

Molecular Formula: $C_{17}H_{19}F_3N_6O$ (Upadacitinib)

Molecular weight: 380.375(Upadacitinib)

Melting point:16-19^{0}c (61-66^{0}F)

The RP-HPLC Technique was used for the development and validation of drug, Upadacitinib. Basing on the literature survey, no other method was found for the estimation of upadacitinib pharmaceutical dosage forms. So it is a proposed method for development and validation as per ICH guidelines.

EXPERIMENT:

REAGENTS AND CHEMICALS:

The drug sample was taken from Ankur pharmacy, Bengaluru. The solvents and chemicals were used for the study, potassium hydrogen phosphate, sodium hydroxide, methanol, acetonitrile were HPLC grade and milli-Q grade water.

INSTRUMENTATION:

The HPLC system 2965, empower 3 software and UV (232nm) detector, Isocratic pump mode, sample injector and column Inert sustain C 18 (150×4.6) mm ,5µ respectively. Electronic Balance, Sonicator (ultra-sonic cleaner power sonic 420), pH meter, Water bath and other glassware used for the present investigation.

CHROMATOGRAPHIC CONDITIONS:

Column: Inert sustain C 18 (150 × 4.6) mm,5 µ

Pump mode: 1.0 ml/ min

Detection: UV,232 nm

Column oven temperature:35^{0}C

Injection volume:20µL

Run time: 10 mins

PREPARATION OF BUFFER:

About 2.72 g of potassium dihydrogen phosphate is weighed and dissolved in 1000 ml of milli Q water. The pH is adjusted at 6.8 with 0.2N Sodium hydroxide solution. The solution is filtered through 0.45µ membrane filter paper.

PREPARATION OF 0.2N SODIUM HYDROXIDE SOLUTION:

0.8 g of sodium hydroxide pellets are weighed and dissolved in 100 ml of water and mixed well.

PREPARATION OF DILUTED ORTHO PHOSPHORIC ACID SOLUTION:

2 ml of ortho phosphoric acid is diluted to 20 ml with water and it is mixed thoroughly.

PREPARATION OF SOLEVENT MIXTURE:

Prepared a degassed mixture of acetonitrile and methanol in parts of 200 ml and 100 ml respectively.

PREPARATION OF MOBILE PHASE:

The degassed mixture of Buffer and solvent mixture is prepared in the ratio of 65:35 v/v.

PREPARATION OF DILUENT-1:

A required volume of degassed mixture of water and acetonitrile in the ratio of 30:70 v/v prepared.

PREPARATION OF DILUENT-2

Water is used as diluent-2.

PREPARATION OF BLANK:

5 ml of diluent-1 is diluted to 50 ml with water and shaken well.

PREPARATION OF SOLUTIONS:

STANDARD SOLUTION-1:

About 50 mg of upadacitinib working standard is taken and transferred into a 100 ml clean and dry volumetric flask. About 70 ml of diluent -1 is added and sonicated to dissolve completely. Then it is diluted to volume with diluent -1 and mixed well. Further diluted 6 ml of above solution to 100 ml with diluent-2 and mixed well. Filter the solution through 0.45µ Millipore pvdf membrane filter paper presaturated with about 5 ml of diluent-2 followed by discarding about 8 ml of the initial solution.

STANDARD SOLUTION-2:

55 mg of upadacitinib is weighed and transferred into a 100 ml clean, dry volumetric flask. About 70 ml of diluent-1 is added and sonicated to dissolve completely. This is furtherly diluted by adding 6 ml of above solution to 100 ml with diluent-2 and mixed well. The solution is filtered through 0.45µ Millipore pvdf membrane filter paper presaturated with about 5 ml of dilutent-2 followed by discarding about 8ml of the initial solution, now concentration of upadacitinib is about 27-33 ppm.

SAMPLE SOLUTION:

10 Tablets is taken into a suitable is taken butter paper and folded it, broken the tablet into coarser fragments with the help of pestle. Carefully transferred the whole amount of the fragments quantitatively into a 500 ml volumetric flask. About 50 ml of diluent-2 is added and sonicated at room temperature with intermittent vigorous shaking at every 2 minutes interval for about 10 minutes. About 85 ml of acetonitrile is added, followed by 215 ml of diluent-1 and uniformly mixed it. A suitable magnetic bead is placed into the volumetric flask and stirred at appropriate rpm for about 30 minutes. Removed the magnetic bead by washing with 20 ml of diluent and sonicate the solution about 60 minutes at room temperature with intermittent shaking at about every 5 minutes interval. Diluted to volume about 1 cm below the mark with diluent-1 and mixed thoroughly. Centrifuged a portion of the solution at about 8000 rpm for about 10 minutes. Diluted 5 ml of the clear supernatant solution to 50 ml with diluent-2 and mixed well. Filter the solution thoroughly with 0.45µ Millipore pvdf membrane filter paper presaturated with about 5 ml of diluent-2 followed by discarding about 8 ml of the initial solution concentration is 30 ppm.

RESULT AND DISCUSSION:

METHOD VALIDATION:

The method validation is a procedure, which performs the all functions like method development, specificity, linearity, accuracy, precision, robustness and system suitability studies.

METHOD DEVELOPEMENT:

The method development is performed with 2.72 g of potassium dihydrogen phosphate in 1000 ml of water and adjusted the pH at 6.8 with diluted sodium hydroxide solution by using Isocratic, degassed mixture of buffer and acetonitrile, which was analyzed by Inert sustain C 18 (150×4.6) mm,5µ column with flow rate of 1.0 ml/min 232 nm wavelength. Column oven temperature 35^0c, run time 10 mins mobile phase is degassed mixture of buffer and solvent mixture in the ratio 70: 30 v/v. Filtered through 0.45µ Millipore pvdf membrane filter paper.

SPECIFICITY:

From the diluents, placebo chromatograms, it is concluded that no peak is observed at the retention time of upadacitinib peak. Therefore, based on above observations, it can be concluded that there is no interference due to diluents and placebo. On above observations, it can be concluded that there is no interference due to diluents and placebo for assay of upadacitinib in upadacitinib extended release tablets. Hence the method is specific.

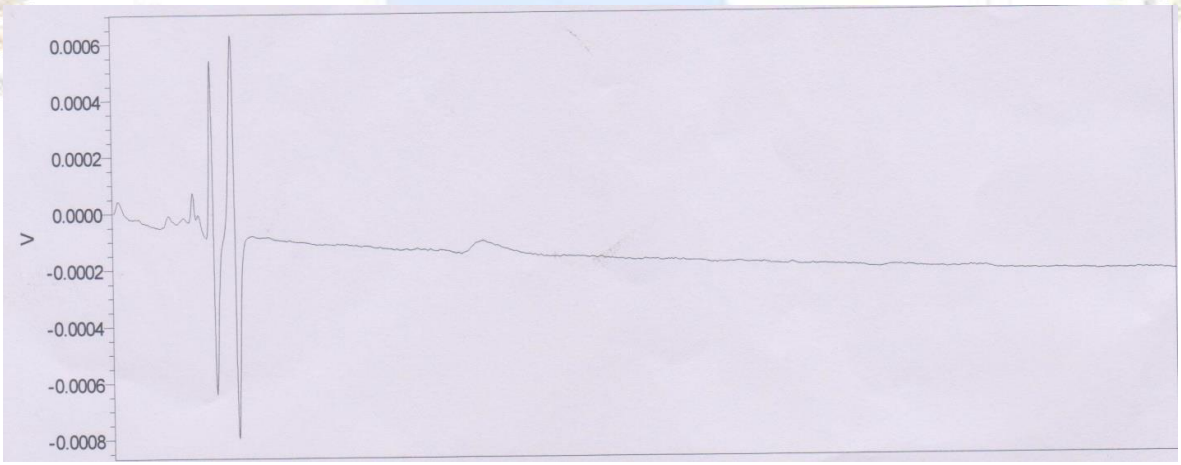


Fig.1 : Diluent chromatogram for specificity.

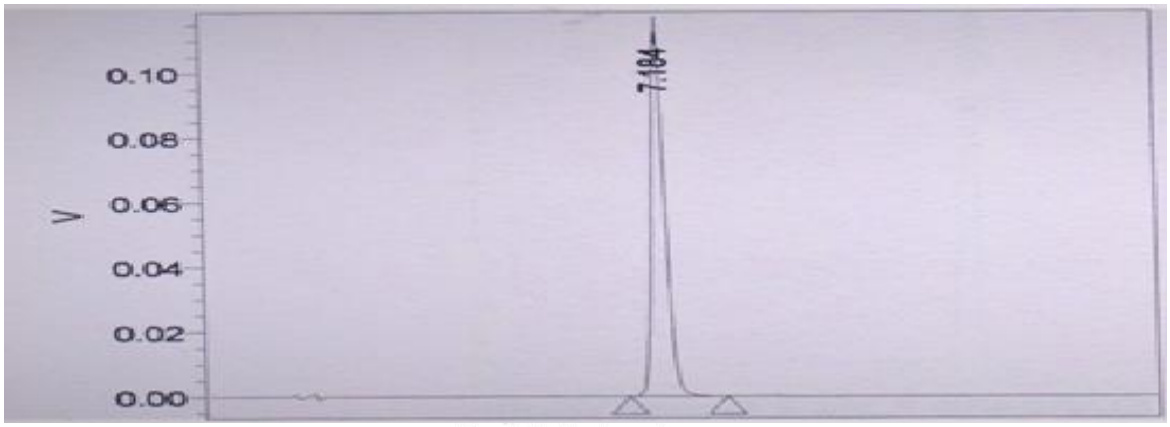


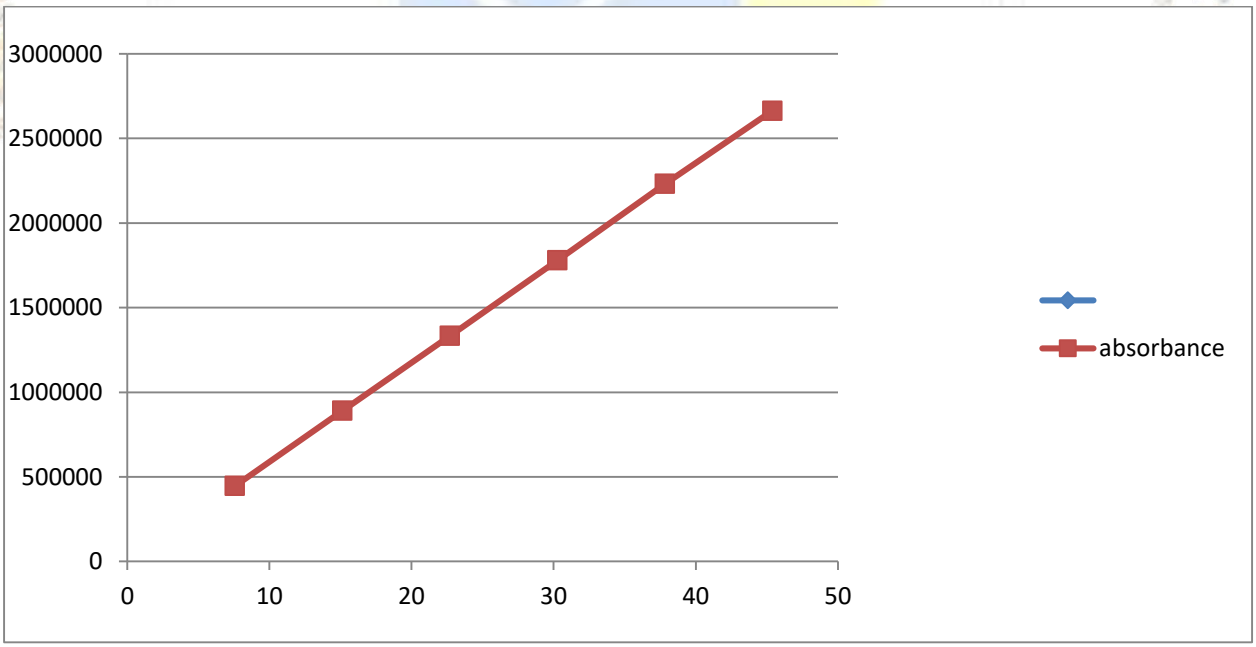
Fig .2: Standard chromatogram for specificity.

Fig.3 : placebo chromatogram for specificity

Conclusion: No interference was observed.

LINEARITY:

The linearity study is done by injecting the different concentrations (25%-150%) of the drug from the linearity curve. The correlation coefficient is found to be less than 1.0. The results are incorporated in the given table. The linearity plots are shown in the fig. 4.



s.no	Linearity level	Concen- tration	Area-1	Area-2	mean area
1	25%	7.566	446619	446676	446648
2	50%	15.131	890463	890323	890393
3	75%	22.697	1333839	1333150	1333495
4	100%	30.263	1781003	1777885	1779444
5	125%	37.829	2230906	2231683	2231295
6	150%	45.394	2662930	2662930	2663547

Conclusion: Correlation coefficient is found 0.99 within the limits.

SYSTEM PRECISION:

20µL of standard solution -1 is injected in 5 replicate injections into the chromatograph, then recorded the chromatograms and measured the peak areas.

SYSTEM PRECISION FOR UPADACITINIB:

The column efficiency is determined for upadacitinib peak from the first injection of the standard solution-1 is not less than 2000 plate counts and the tailing factor for the same peak should be between 0.8 to 2.0.

%RSD for the peak area of upadacitinib from the five replicate injections of standard solution- 1 should be not more than 2.00%.

INJECTION	Rt	AREA
1	7.184	1745938
2	7.181	1746252
3	7.183	1742866
4	7.178	1743489
5	7.174	1742234

6	7.174	1665434
MEAN		1731036
%RSD		1.86

Conclusion: % RSD of standard solution-1 of 6 replicate injections 1.86.

METHOD PRECISION FOR UPADACITINIB:

Six sample solutions are prepared individually using single batch and injected the solutions into HPLC as per methodology.

SAMPLE NO	AREA
1	1789641
2	1787216
3	1779769
4	1783821
5	1788236
6	1790345
MEAN	1785774
SD	0.27
% RSD	0.27

Conclusion: From the above results, it can be concluded that the test method is precise.

% RSD of six different sample injections is 0.27, within limits.

ROBUSTNESS STUDY OF UPADACITINIB:

Robustness is a parameter, it has been evaluated in validation studies of analytical method. It is analytical procedure to produce unbiased results in the presence of experimental conditions.

Robustness values for Upadacitinib:

chromatographic conditions	Rt	theoretical plates	tailing factor
1.0 ml/min,232 nm 35^{0}c pH 6.8 buffer: solvent mixture (65:35) v/v	6.57	6348	1.12
flow rate: 0.9 ml/min	7.25	6589	1.15
flow rate: 1.1 ml/ min	5.97	5744	1.13
temparature:30^{0}c	6.88	5545	1.18
temparature:40^{0}c	6.39	6385	1.12
wave length:227 nm	6.55	6091	1.15
wave length:237 nm	6.57	5990	1.15
pH: 6.6	6.73	6060	1.15
pH:7.0	6.71	6104	1.14
organic: buffer: acetonitrile (67:33)	9.75	7420	1.02
organic: buffer: acetonitrile (63:37)	6.58	6868	1.03

Conclusion: Due to Deliberate changes in this method, which is not affected to analytical method. Hence the method was Robust.

ACCURACY:

Accuracy is the drug at this level is about 50%, 100%, 150% of test concentration.

Recovery should be in the range of 98.0% to 102.0% with %RSD NMT 2.0%.

Drug name	Concen - tration%	amount added	amount found	% recovery	statical analaysis
upadacitinib	50%	74.07	73.91	99.80	mean-99.74
upadacitinib	100%	148.43	148.48	100.04	SD-0.59
upadacitinib	100%	295.58	292.29	98.89	% RSD-0.59
upadacitinib	150%	441.74	442.68	100.21	% RSD-0.59

Conclusion: % Recovery of 50%,100%,150% were found within the limits.

STANDARD AGREEMENTS:

20µL of standard solution-2 is injected in single into the chromatograph, recorded the chromatograms and measured the upadacitinib peak area. The standard agreement between standard solution-1 and standard solution -2 should be in the range of 0.98 to 1.02.

CALUCULATION OF THE STANDARD AGREEMENT:

$$\frac{\text{Average peak area of five injections of standard solution-1}}{\text{Peak area of standard solution-2}} \times \frac{\text{weight of standarad-2(mg)}}{\text{weight of standard-1(mg)}}$$

Content of upadacitinib =

$$\frac{A_T}{A_S} \times \frac{D_S}{D_T} \times 100$$

A_T = Average area counts of upadacitinib peak in the chromatogram of sample solution

A_S = Average area counts of upadacitinib peak in the chromatogram of standard sotuion-1 as obtained under suitability

D_T= Dilution factor of sample solution (tablet/ dilution)

P =Percent potency of upadacitinib of working standard used

% Labeled amount = $\frac{\text{content of upadacitinib (mg/tablet)}}{\text{Lable claim, in mg per tablet}} \times 100$

CONCLUTION:

The method is found to be simple, valuable, accurate and rapid to determine the upadacitinib from pharmaceutical dosage forms. By using different parameters like specificity, linearity, accuracy, precision and system suitability, the method was validated as per ICH guidelines. The method is considered to have following qualities such as less R_t, selectivity and accuracy.

The validated method is suitable for Quality control and determination of Upadacitinib in pharmaceutical dosage forms.

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