BIO ANALYTIC AND ANALYTIC METHOD QUANTITATIVE TO TUCATINIB ESTIMATION IN PURE AND PHARMACEUTICAL DOSAGE FORM

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Abstract

A simple one, accurate and precise method for estimating tucatinib in tablet dosage form was developed on symmetry C_{18} column (150x4.6mm, 3.5µ) using buffer and acetonitrile 40:60 pumped through a column with 1ml/min flow rate 0.1 percent formic acid of the buffer used in this method. The run time was 6 min in assay method and 12 min in bio analytical method. Reference standard and sample solutions were prepared by dissolving firstly in acetonitrile and diluted with diluents (mobile phase). Cisplatin was used as internal standard in bio analytical technique. These assay and bio analytical methods establishgood linearity results (R^2-0.999) with an optimized wavelength of 220nm. The technique was validated in assay method with respect to specificity, linearity, accuracy, robustness, LOD, LOQ, method precision, intermediate precision, degradation and in bio analytical method the technique was validated regarding specificity, linearity, recovery, matrix factor, precision, accuracy and stability.

Key Words: Tucatinib, HPLC, development, LC-MS/MS, rat plasma.

1. Introduction

Tucatinib [1] sold under the name Tukysa, may be a small HER2 molecule inhibitor for HER2 positive carcinoma [2-4] treatment. It was developed by Array Biopharma and licensed for Cascadian therapy (formerly oncothyreon, later part of Seattle genetics). The recommended dose of tucatinib is 300 mg dialy taken twice by mouthwith trastuzumab (at standard dosage) and capecitabine (1000 mg/m^2 twice daily on days 1-14 of a 21- day cycle) are unacceptable until disease progression or toxicity. Common side effects include diarrhea [5-6] palmar-plantar erythrodysesthesia [7-8] (burning or tingling discomfort within the hands and feet), nausea, fatigue [9-10], hepatotoxicity [11-12] (liver damage), vomiting, stomatitis [13-14] (inflammation of the mouth and lips), decreased appetite, abdominal pain, head ache, anemia [15] and rash. Pregnant or breast feeding women should not take tucatinib as it will harm the developmentof a foetus or neonate. Tucatinib may be a kinase inhibitor [16] indicated in combination with trastuzumab and capecitabine for the treatment of adults with unrespectable or metastatic HER2 positive carcinoma, including those with brain metastases [17-18] who have received one or more adverse anti-HER2 based regimens.

2. EXPERIMENTAL STUDY

2.1 Chemicals and reagents

Acetonitrile, formic acid, water and methanol were producedfrom Merck (India) Ltd, Worli, and Bombay, India. API of tucatinib was procured from Zydus Cadila, Ahmedabad.

2.2 Instrumentation

2.2.1 HPLC Conditions

For the waters alliance e-2695 was used the liquid chromatography system consisting of quaternary pump, PDA detector 2996 and chromatographic software Empower 2.0.

2.2.2 LC-MS/MS Conditions

Process chromatography involves the column of symmetry C_{18} (150x4.6mm, 3.5 µ) with ambient temperature. An isocratic elution of 40:60 of ACN and Formic acid 0.1 percent was used as movable phase. Flow of 1 ml/min with dose volume 20 µl is used in LC-MS/MS. Forced degradation study was connected into mass spectrophotometer of conditions, a splitter was placed front of the source of ESI allowsonly 35 percentof thateluents enter. The conditions of the quality MS operating source tucatinib scan in positive ESI mode scanhave been optimised as follows. The voltage of the fragmenter was set at 80V, the capillary at 3000V, the skimmer at 60V, the drying and nebulizing gas (45psi) was used as nitrogen. Nitrogen gas which was highly filtered was used as collision gas.

2.3 Chromatographic conditions

Chromatographic separation was administered in isocratic mode at temperature employing a symmetry C_{18} (150x4.6mm, 3.5 µ) column. The mixture of formic acid 0.1 percent and acetonitrile 60:40 v/v at a flow of 1ml/min was used as a mobile phase. The injection volume was 10 µl and eluents were monitored at 220nm using PDA detector. The run time in assay method was 6 min and in bio analytical method was 12 min. respectively.

2.4 Preparation of stock and dealing standards in assay method

Preparation of ordinary solution: Accurately weigh 50 mg of tucatinib working standard and transformed into volumetric 100ml flask. Added app. 70ml diluents and 15 min sonicised. to

dissolve the component. After 15 min make the mark right with diluents. Further diluted 5 ml of the solution above for 50ml with diluents.

Preparation of specimen solution: Weighed two tablets, and sample crushed like 50mg of tucatinib was taken and transferred to 100ml volumetric flask, 70ml diluents were added and 30mintues sonicated to dissolve the components then diluted with diluents to the mark. Additionally dilute 5ml of the solution set out above with diluents to 50ml and it was filtered through a 0.45µ nylon syringe filter.

2.5 Preparing the stock and dealing standards in bio analytical method

Preparation of ordinary solution: 50ng/ml of tucatinib solution was prepared by diluting the flask with diluents.

Preparation of sample solution: Take 500µl of plasma, 500µl of acetonitrile, 500µl of internal standard and 500µl of standard stock solution in eppendorf tube using a micro pipette and vortexed for 10min. Further centrifuge at 5000 rpm for 20 min and the resulting solution is used for analyzing.

3. RESULTS AND DISCUSSION:

3.1 Method Development and optimisation

The most appropriate isocratic condition to observe tucatinib with C_{18} column symmetry after optimizing the chromatographic conditions for specificity, resolution and retention time and a mobile phase of 0.1 percentHCOOH and ACN with the ratio of 60:40. The chromatogram had risen in back background noise or peaks indicating the tailing effect when a better percentage of the mobile phase was being used. Thus the above mentioned parameters peak was supported eluted a retention time of 4.347 minutesin assay method and 4.204 minutesin bio-analytical method. Table 1 depicts the parameter of chromatographyused for the technique.

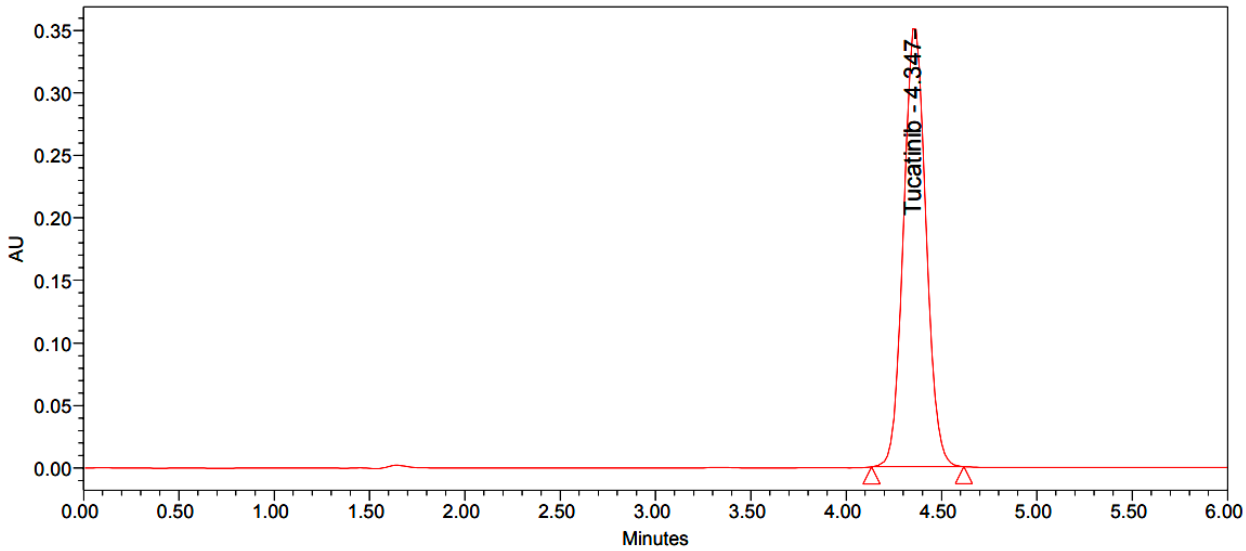


Figure1 Representative tucatinib chromatogram in assay method

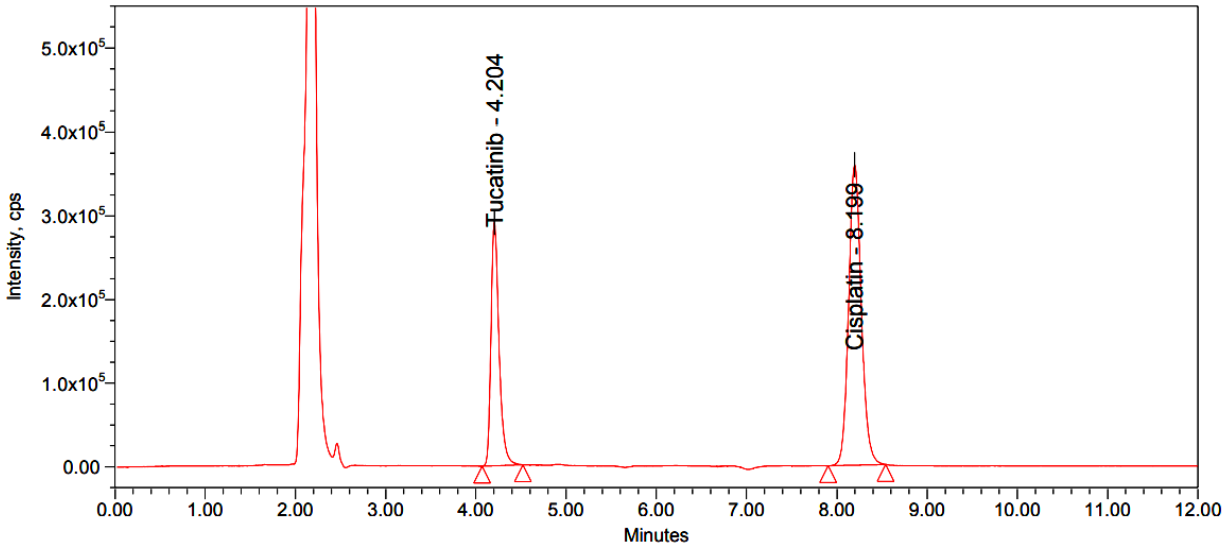


Figure 2Tucatinib representative chromatogram in bio analytical method

Table 1 Assay method of tucatinib

Parameter	Optimization condition
Column	Symmetry C_{18} (150x4.6mm, 3.5µ)
Mobile phase	Acetonitrile+ Formic acid 0.1 percent(40:60)
Flow Frequency	1 ml/min
Volume of Injection	10 µl
Wavelength	220nm
Retention time	4.347 minutes
Run time	6min

Table 2 Bio analytical method of tucatinib

Parameter	Optimization condition
Column	Symmetry C_{18} (150x4.6mm, 3.5µ)
Mobile phase	Acetonitrile+ Formic acid 0.1 percent (40:60)
Flow Frequency	1 ml/min
Volume of Injection	20 µl
Wavelength	220nm
Retention time	Tucatinib- 4.204min Cisplatin (IS)- 8.197min.
Run time	12min

3.2 Validation of Method

A validation of the method in accordance with the validation of analytical procedures provided for by the ICH guidelinesQ2 (R1) and draught industry guidance, analytical procedures and validation of the method.

3.2.1 System precision

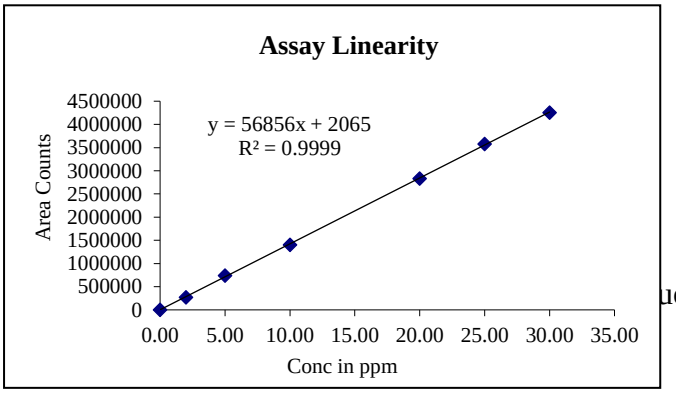
The HPLC and LC-MS/MS it has stabilized the system for 60 min. to insiston stable line. Six standard solutions replicate injections containing standard solution 50µg/ml of tucatinib for assay validation and 50ng/ml of tucatinib for bio analytical validation was assessed to ascertainsuitability of the system. The theoretical number plate count was observed as 6527, tailing factor was 1.04 respectively. The values of these parameters were found to be within the suitable limit.

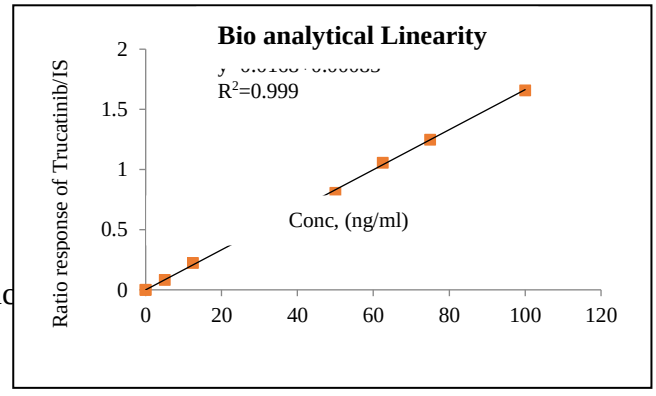
3.2.2 Linearity and range

Linearity of the assay and bio analytical techniques was evaluated by preparing a typical solution containing 50µg/ml and 50ng/ml respectively. Sequential dilutions were performed to the given solutions at 10, 25, 50, 100, 125, 150% of the target concentrations in assay method and 10, 25, 50, 75, 100, 125, 150, 200% of the target concentrations in bio analytical method respectively. These were injected and measure the peak areas. Plot a calibration curve by focusing onthe X-axis and Y-axis peak areas respectively. In both methods the correlation coefficient was observed as 0.999.

Table 3Linearity Data

Linearity	Assay Validation Linearity		Bio analytical Validation Linearity	
	Concentration (µg/ml)	Area of the Peak	Conc. (ng/ml)	Area Ratio Response
1	5	273054	5	0.083
2	12.5	743514	12.5	0.222
3	25	1406209	25	0.433
4	50	2834505	37.5	0.628
5	62.5	3578517	50	0.805
6	75	4255617	62.5	1.055
7	-	-	75	1.249
8	-	-	100	1.658
Slope	56856.33		0.0168	
Intercept	2065.71		0.00085	
CC	0.9999		0.9999	





Limit of detection and quantification minimum concentration level at which the analyte are often reliably detected, quantified by using the quality formulas (3.3 times σ/s and 10 times σ/s for LOD and LOQ respectively. LOD and LOQ values of tucatinib in assay method is 0.05µg/ml and 0.5µg/ml respectively. LOD and LOQ values for tucatinib in bio analytical method is 0.05ng/ml and 0.5ng/ml respectively.

3.2.4 Accuracy and precision

In assay method accuracy decided by recovery studies which were administered in three different concentration levels (50%, 100% and 150%). APIs with concentration 25, 50 and 75µg/ml of tucatinib was prepared. As per the test method the test solution was injected to three preparations each spike level and therefore the assay was performed. The recovery values were found to be within the range of 98-101%.

Method precision was investigated by the analysis of six separately prepared samples of an equivalent batch. From these six separate samples, solution was injected and therefore the peak areas obtained wont to calculate mean and percentage of RSD. This method has been found to be accurate since the percentage of RSD is less than 2%.

The inter run and accuracy have been evaluated in bio analytical method by pooling all individual assay results of five separation batch internal replicate control runs analyzed over found different days. The inter run precision percentage CV was <5 percent and the inter run precision values for tucatinib were between 85-115. It was clear from the data that precision and accuracy are precise and accurate.

Table 5 Accuracy results of tucatinib in assay

% of target concentration	Tucatinib (% recovery)	Tucatinib (% RSD)
50%	99.9	1.19
100%	100.6	0.6
150%	99.3	0.33

Table 6 Method precision results of assay

Aanalyte	Amount present	% Assay	% RSD of assay
Tucatinib	50mg	99.6	0.4

Table 7insideand between tucatinib run precision and accuracy

Nominative conc. (ng/ml)	Within run			Between run		
	Average (ng/mL)	Precision (%CV)	Accuracy	Average (ng/mL)	Precision (%CV)	Accuracy
5	5.231	0.12	100.5	5.326	0.32	100.3
25	25.412	0.36	100.1	25.247	0.41	99.9
50	50.269	0.57	99.8	50.132	0.22	100.6
75	75.029	0.15	100.3	75.058	0.19	110.2

3.2.5 Robustness

Robustness of the technique was found to draw in RSD should be 2%. Slightly variations were exhausted the optimized method parameters like flow (±0.2ml/min), organic content at mobile phase (±10%). Results have been tabulated in table 8.

Table 8 Robustness results

Drug name	Flow plus (1.2ml/min)	Flow minus (0.8ml/min)	Org plus (44:56)	Org minus (36:64)
	% RSD			
Tucatinib	0.38	0.11	0.75	1.56

3.2.6 Recovery

Tucatinib mild, medium and high quality management standards have been prepared for assessing rehabilitation, and hence areas collect for evaluate from the same concentration stage of a batch with accuracy and precision produced on an equal day. Tucatinib average recovery was 98.45 percent and precision is 1.06 percent this means that the extraction efficiency of tucatinib.

3.2.7 Matrix effect

For tucatinib, the suppression/enhancement of the ion percentage CV of the signal was calculated to be 1.1 percent at the MQC stage. It indicates that the effect of the matrix on analyte ionization occurs beyond the appropriate guidelines.

3.2.8 Carryover

Systematic error which will effect the measured value of the sample is named carryover. Sample carryover on a LC-MS/MS system configured with Waters Alliance was evaluated using the subsequent procedure. A system blank injection of 20µl acetonitrile and formic acid 0.1 percent (40:60) was made onto water ZSpray triple quadrupole mass detector using flow injection analysis. From this we will say it does not impact the quality of the precision of the method submitted. Sample carryover was it expressed as each side carryover and nL carryover. The sample carryover results are tabulated within the following table.

Table 9 Carryover results of tucatinib

Concentration	%CV carryover of tucatinib
Blank	0.00
LLQC	0.26
ULQC	0.55

3.2.9 Integrity with Dilution

The dilution quality experiment was performedwith the aim of validating the dilution proposed to be conducted at higher concentrations of analytes over the upper limit of quantification (ULOQC) that can be found inthe actual sample analysis. Analyte spiking stock solution was spiked to urge concentration like 3 times of ULOQC in blank plasma and

diluted with blank plasma to urge 1/5 and 1/10 concentrations of the spiked sample or as per required. Calibration standards and 6 aliquots were processed and analyzed for each of the diluted samples, as described in sample preparation procedure. The accuracy and precision of the QC's dilution integrity should be about 15%.

3.2.10 Stability

To check the stability of tucatinib, stock solution was prepared and stored in fridge ata temperature 2-8°C. Compare the freshly prepared stock solution with the stock solution stored before 24hrs. From this we observed a change of tucatinib was 1.12%. It indicates that stock solutions are stable up to 24hrs. Stabilities of the bench top and auto sampler at LQC and HQC levels were observed. At temperature tucatinib was stable in 24 hours plasma and 24hrs in auto sampler at 20°C auto sampler. From this it hasbeen verified that regular freezing and thawing of plasma samples spiked with tucatinib did not affect their stability at low and high concentration levels. It was clear from long-term stability that tucatinib was stable at the temperature of storage of -30°C until 24hrs.

Table 10 Bio analytical stability results of tucatinib

Stability experiments		Spiked plasma conc (n=6, ng/ml)	Conc. measurements (n=6, ng/ml)	%CV (n=6)
Bench top stability	LQC	25	25.364	0.24
	HQC	75	75.128	0.16
Auto sampler stability (24 Hrs)	LQC	25	25.254	0.38
	HQC	75	75.106	0.05
Freeze thaw stability	LQC	25	25.429	0.45
	HQC	75	75.325	0.11
Wet extract stability (18 Hrs)	LQC	25	25.348	0.37
	HQC	75	75.429	0.29
Dry extract stability (18 Hrs)	LQC	25	25.169	0.56
	HQC	75	75.648	0.30
Long term stability (Day 28)	LQC	25	25.039	0.15
	HQC	75	75.248	0.28
Short term stability	LQC	25	25.364	0.47
	HQC	75	75.481	0.33

In assay method stability of ordinary and sample solutions are studied from initial to 24hrs stored at RT. They were injected at different time intervals and difference between initial to 24 hrs percentage of assay wasn't quite 2%. There is no effect in storage conditions of tucatinib drug.

Table 11 Assay stability results of tucatinib

Stability	% of lable claim Tucatinib	% deviation of Tucatinib
Initial	100.9	0.00
6 Hrs	100.7	-0.20
12 Hrs	100.4	-0.50
18 Hrs	99.4	-1.49
24 Hrs	98.9	-1.98

3.2.11 Forced degradation

Forced degradation conditions containing acidic, basic, peroxide, hydrolysis, reduction and thermal stress were studied in 0.1N and 1N concentration levels.

Table 12 Forced degradation results of tucatinib

Degradation	% lable claim	% degradation
Control degradation	100.1	-0.1
Acid degradation	67	33.1
Alkali degradation	68.6	31.4
Peroxide degradation	66.6	33.4
Reduction degradation	67.1	32.9
Thermal degradation	66.7	33.3
Hydrolysis degradation	66.5	33.5

3.3 Pharmacokinetic study

The method is implementedappliedto quantify tucatinib concentration in six different rats following administration of the50 mg tablet with tucatinib as an oral therapy, under fasting condition. After injecting the drug samples into rat body collect the samples different times,such as 0.5, 2.5, 4.5, 6.5, 8.5, 10.5, 12.5, 14.5 and 16.5 min respectively from the rat body. Then as per test method sample is ready and injected into the chromatographic system and record the values. The pharmacokinetic parameterstestedwere C_{max} (absolute drug concentration observedthroughout the study), AUC_{0-12} (area under the plasma concentration-time curve estimated, 30min using the trapezoidal rule), t_{max} (time to absolute drug concentration observed), K_{el} (apparent first-order terminal first order velocitydetermined from the semi-log plot of the plasma concentration plot vs time curve) and $t_{1/2}$ (final half life as calculated by $0.693/K_{el}$quotient). The ratio of test/reference for C_{max}, AUC_{0-12} and AUC were 84.26 and 92.31 respectively and found to be within the acceptable limit of 80%-125%.

Table 13 Pharmacokinetic parameters of Tucatinib

Parameter: Pharmacokinetics	Tucatinib
AUC_{0-t} (h/ml ng)	390
C_{max} (ng/ml)	42.9
$AUC_{t-\infty}$ (h/ml ng)	47
$AUC_{0-\infty}$(h/ml ng)	436
$T_{1/2}$	8.5
t_{max} (h)	8.5

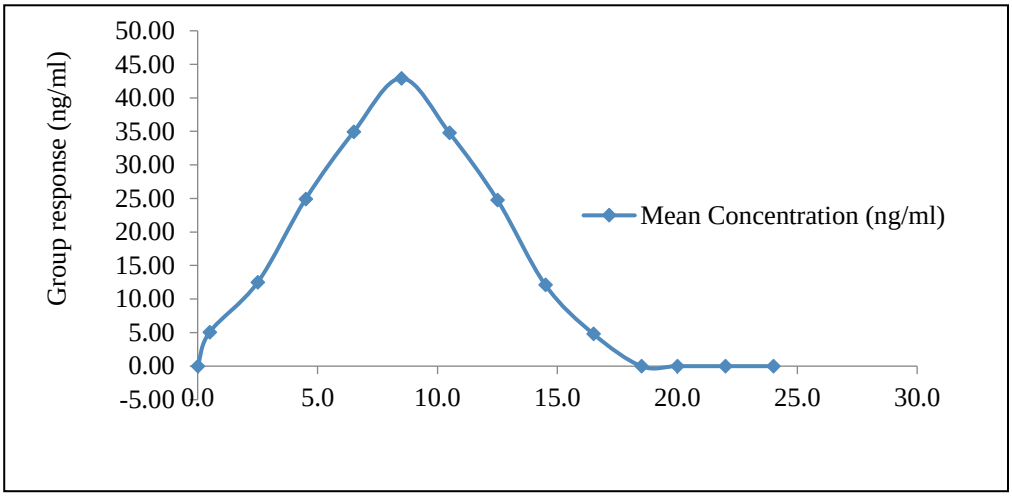


Figure 4 Recovery plot for tucatinib

4. CONCLUSION

In this study a completely unique, simple, rapid, economical and sensitive HPLC method for determining tucatinib inapiand tablet formulation formhas been built. Design of the method is desirable as it is cost-effective, accessible, sensitive, reliable and reproducible with shorter run time. These properties are important when an outsized number of samples are to be analyzed. The method proposed could easily applied to routine analysis and pharmaceutical formulations of tucatinib in quality control laboratories with none preliminary separation. The most sensitive HPLC-ESI-MS/MS decision tucatinib plasma in rat process has been developed and validated for the first time.The currently developed method is easy, efficient, fast, rugged, reproducible bio analytical method and may be utilized in pharmacokinetic studies and to see the investigated analyte in body fluids.

CONFLICT OF INTEREST

The author announces there were no conflicting interests.

5. REFERENCES

1. World health organization,2016. International nonproprietary names for pharmaceutical substances (INN). Recommended INN: list 75. WHO drug information.30 (1) 161.
2. Martin M, Lopez-Tarruella S. 2018. Emerging therapeutic actions for HER2 positive breast cancer.American society of clinical oncology educational book.American society of clinical oncology.Annual meeting.35, e64-70.
3. Song JK, Bae JM. 2013. Citrus fruit intake and breast cancer risk.A quantitative systematic review.Journal of breast cancer.16 (1), 72-76.
4. Tonelli M, Connor Gorber S, Joffres M, Dickinson J, Singh H, Lewin G. 2011. Recommendations on screening for breast cancer in average risk women aged 40-74 years. CMAJ. 183 (17), 1991-2001.

5. Smalley W, Falck-ytter C, Carrosco-labra A, Wani S, Lytvyn L, Falck-ytter Y. 2019. AGAclinical practice guidelines on the laboratory evaluation of functional diarrhea and diarrhea predominant irritable bowel syndrome in adults (IBS-D). Gastroentorology. 157 (3), 851-854.
6. Moon C, Zhang W, Sundaram N, Yarlagadda S, Reddy VS, Arora K, Helmrath MA, Naren AP. 2015. Drug induced secretory diarrhea. A role for CFTR.Pharmacological research. 102, 107-112.
7. Juergen-Barth. 2004. Letter to the editor-5-FU-induced palmar-plantar erythrodyesthesia a hospital developed antidot. Journal of organic pharmacy practice.10, 57.
8. Bashashati, Mohammad, McCallum, Richard W. 2014. Neurochemical mechanisms and pharmacologic strategies in managing nausea and vomiting related to cyclic vomiting syndrome and other gastrointestinal disorders. European journal of pharmacology. 722, 79-94.
9. Zielinski M R. Systrom D M, Rose N R. 2019. Fatigue, sleep, autoimmune and related disorders. Front immunol. 10, 1827.
10. Nijrolder iris, Van der Windt, Danielle, De Vries, Henk, Van der Horst, Henriette. 2009. Diagnosis during follow-up of patients presenting with fatigue in primary care.Canadian medical association journal.181 (10), 683-687.
11. Wallace JL. 2004. Acetaminophen hepatotoxicity. NO to the rescue. Br. J. Pharmacol. 143 (1), 1-2.
12. Jaeschke H, Gores GJ, Cederbaum AI, Hinson JA, Pessayre D, Lemasters JJ. 2002. Mechanisms of hepatotoxicity. Toxicol. Sci. 65 (2), 166-176.
13. Brocklehurst P, Tickle M, Glenny AM, Lewis MA, Pemberton MN, Taylor J, Walsh T, Riley P, Yates JM, Brocklehurst P.2012.Systematic interventions for recurrent aphthous stomatitis. The Cochrane data base of systematic reviews. 9 (9), CD005411.
14. Solomon LW. 2008. Chronic ulcer active stomatitis. Oral diseases.14 (5), 383-389.
15. Allen RP, Auerbach S, Bahrain H, Auerbach M, Earley CJ. 2013. The prevalence and impact of restless legs syndrome on patients with iron deficiency anemia.Am J Hematol.88 (4): 261-264.
16. Janne, Pasi A, Gray, Nathanael, Settleman, J. 2009. Factors underlying sensitivity of cancers to small molecule kinase inhibitors. Nature reviews drug discovery. 8 (9),709-723.
17. Caponnetto S, Draghi A, Borch TH, Nuti M, Cortesi E, Svane IM, Donia M. 2018. Cancer immunotherapy in patients with brain metastases. Cancer immunology, immunotherapy. 67 (5), 703-711.
18. Stark AM, Stohring C, Hedderich J, Held-Feindt J, Mehdorn HM. 2011. Surgical treatment for brain metastases, prognosis factors and survival in 309 patients with regard to patient age. Journal of clinical neuroscience.18 (1), 34-38.