Method Development and Validation for The Estimation of Anti-Cancer Drug Dasatinib in Marketed Pharmaceutical Dosage Forms By RP-HPLC

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ABSTRACT

A simple, rapid, specific and accurate reverse phase high performance liquid chromatographic method has been developed for the validated of Dasatinib in bulk as well as in marketed pharmaceutical dosage form. This separation was performed on a Symmetry ODS (C18) RP Column, 250 mm x 4.6 mm, 5µm column with Acetonitrile, Methanol and 0.1% OPA in the ratio of 55:35:10% v/v/v as mobile phase at a flow rate of 1.0 mL min−1 with UV detection at 235 nm; the constant column temperature was Ambient. The run time under these chromatographic conditions was less than 6.0 min. The retention time of Dasatinib was found to be 2.631min. The calibration plot was linear over the concentration range of 6–14 µg mL−1 with limits of detection and quantification values of 0.8 and 0.24ng mL−1 respectively. The mean % assay of marketed formulation was found to be 99.79%, and % recovery was observed in the range of 98-102%. Relative standard deviation for the precision study was found <2%. The developed method is simple, precise, specific, accurate and rapid, making it suitable for estimation of Dasatinib in bulk and marketed pharmaceutical dosage form dosage form.

KEY WORDS:

Dasatinib, RP-HPLC, Validation, Accuracy, Precision, Robustness, ICH Guidelines.

INTRODUCTION

Dasatinib is an orally available multikinase inhibitor indicated for the treatment of Philadelphia chromosome (Ph)-positive leukemias. Ph is a chromosomal abnormality found in patients with chronic myelogenous leukemia (CML) and acute lymphocytic leukemia (ALL), where the ABL tyrosine kinase and the breakpoint cluster region (BCR) gene transcribe the chimeric protein BCR-ABL[1]. BCR-ABL is associated with the uncontrolled activity of the ABL tyrosine kinase and is involved in the pathogenesis of CML and 15-30% of ALL cases. Dasatinib also inhibits a spectrum of kinases involved in cancer, including several SRC-family kinases. Unlike [Imatinib], another tyrosine kinase used for the treatment of CML and Ph-positive ALL, Dasatinib inhibits the active and inactive conformations of the ABL kinase domain[2]. Also, mutations in the kinase domain of BCR-ABL may lead to relapse during Imatinib treatment. Since Dasatinib does not interact with some of the residues involved in those mutations, the use of this drug represents a therapeutic alternative for patients with cancers that have developed Imatinib-resistance[3]. The use of Dasatinib was first approved by the FDA in 2006. The IUPAC Name of Dasatinib is N-(2-chloro-6-methyl phenyl)-2-[[6-[4-(2-hydroxy ethyl) piperazin-1-yl]-2-methyl pyrimidin-4-yl] amino]-1, 3-thiazole-5-carboxamide.
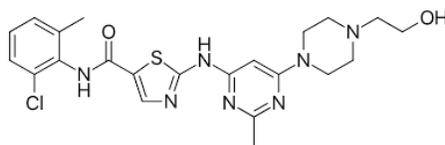
The Chemical Structure of Dasatinib is follows:

Fig-1: Chemical Structure of Dasatinib

There are several HPLC methods[32-35] either in pharmaceutical products or biological samples reported in the literature for determination of Dasatinib alone, with other agents. Some of these methods used isocratic elution to separate the tested analytes.

The present study is aiming to develop and validate a simple, sensitive, rapid, and economic and isocratic HPLC method[4] for the determination of Dasatinib on the same chromatographic run without the need for any costly treatment.

MATERIALS AND METHODS

Instruments Used

Table 1: List of Instrument used

Instruments/Equipment/Apparatus
HPLC with Empower2 Software with Isocratic with UV-Visible Detector (Waters).
T60-LAB INDIA UV – Vis spectrophotometer
Electronic Balance **(SHIMADZU ATY224)**
Ultra Sonicator **(Wensar wuc-2L)**
Thermal Oven
Symmetry ODS RP C_{18},5µm, 15mm x 4.6mm i.d.
P^H Analyzer **(ELICO)**
Vacuum filtration kit **(BOROSIL)**

Chemicals / Reagents Used

Table 2: List of Chemicals used

Name	Specifications		Manufacturer/Supplier
	Purity	Grade	
Doubled distilled water	99.9%	HPLC	Sd fine-Chem ltd; Mumbai
Methanol	99.9%	HPLC	Loba Chem; Mumbai.
Dipotassium hydrogen orthophosphate	96%	A.R.	Sd fine-Chem ltd; Mumbai
Acetonitrile	99.9%	HPLC	Loba Chem; Mumbai.
Potassium dihydrogen orthophosphate	99.9%	A.R.	Sd fine-Chem ltd; Mumbai
Sodium hydroxide	99.9%	A.R.	Sd fine-Chem ltd; Mumbai
Hydrochloric acid	99.9%	A.R.	Loba Chem; Mumbai.
Hydrogen Peroxide	99.9%	A.R.	Loba Chem; Mumbai.

Method Development

Selection of Wavelength

The Standard & Sample Stock Solutions were prepared separately by dissolving standard & sample in a solvent in mobile phase diluting with the same solvent. (After optimization of all conditions) for UV analysis[5]. It scanned in the UV spectrum in the range of 200 to 400nm. This has been performed to know the maxima of Dasatinib, so that the same wave number can be utilized in HPLC UV detector for estimating the Dasatinib. The scanned UV spectrum is attached in the following page,

Sample & Standard Preparation for the UV-Spectrophotometer Analysis

25 mg of Dasatinib standard was transferred into 25 ml volumetric flask, dissolved & make up to volume with mobile phase. Further dilution[6] was done by transferring 0.5 ml of the above solution into a 10ml volumetric flask and make up to volume with mobile phase.

Optimization of Chromatographic Conditions: The chromatographic conditions[7] were optimized by different means. (Using different column, different mobile phase, different flow rate, different detection wavelength & different diluents for sample preparation etc.

Table 3: Summary of Process Optimization

Column Used	Mobile Phase	Flow Rate	Wave length	Observation	Result
Symmetry C_{18}, ODS, Reverse Phase, 250 mm x 4.6 mm, 5µm, Column.	Methanol: Acetonitrile = 40: 60	1.0ml/min	235nm	Very Low response	Method rejected
Symmetry C_{18}, ODS, Reverse Phase, 250 mm x 4.6 mm, 5µm, Column.	Methanol: Acetonitrile= 55: 45	1.0ml/min	235nm	Low response	Method rejected
Symmetry C_{18}, ODS, Reverse Phase, 250 mm x 4.6 mm, 5µm, Column.	Acetonitrile: Water= 50:50	1.0ml/min	235nm	Tailing peaks	Method rejected
Symmetry C_{18}, ODS, Reverse Phase, 250 mm x 4.6 mm, 5µm, Column.	Methanol: Water = 70:30	1.0ml/min	235nm	Resolution was not good	Method rejected
Symmetry C_{18}, ODS, Reverse Phase, 250 mm x 4.6 mm, 5µm, Column.	ACN: Methanol: 0.1% OPA = 70:25:5	1.0ml/min	235nm	Tailing peak	Method rejected
Symmetry C_{18}, ODS, Reverse Phase, 250 mm x 4.6 mm, 5µm, Column.	ACN: Methanol: 0.1% OPA = 60:30:10	1.0ml/min	235nm	Nice peak	Method accepted

Preparation of Mobile Phase:

600ml of HPLC Grade Acetonitrile, 300ml of HPLC Grade Methanol and 100ml 0.1% OPA were mixed well and degassed in ultrasonic water bath for 15 minutes. The solution was filtered through 0.45 µm filter under vacuum filtration[8].

RESULTS AND DISCUSSION

Development of a New Analytical Method:
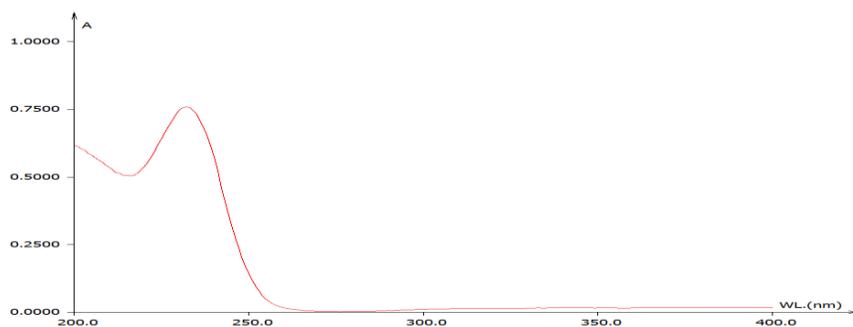
Selection of Wavelength:

Fig 2: UV Spectrum for Dasatinib

Observation: While scanning the Dasatinib solution we observed the maxima at 235nm. The UV spectrum has been recorded on T60-LAB INDIA make UV–Vis spectrophotometer model UV-2450.

Summary of Optimized Chromatographic Conditions:

The Optimum Chromatographic conditions obtained from experiments can be summarized as below:

Table 4: Summary of Optimised Chromatographic Conditions

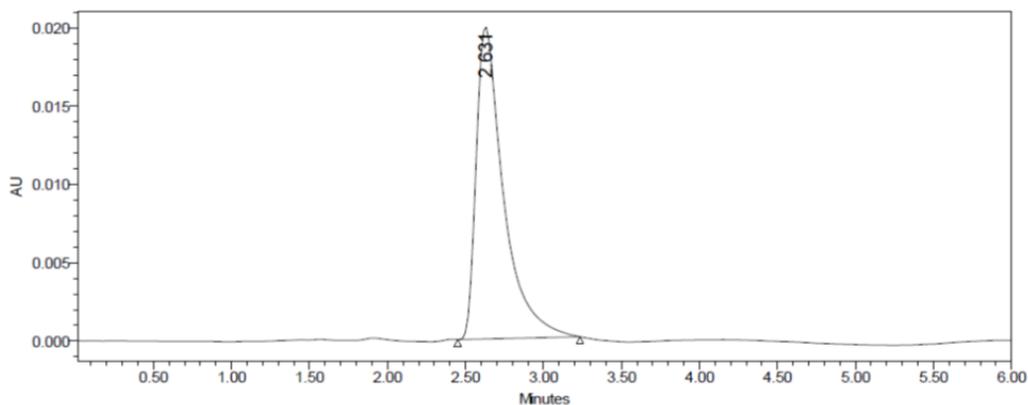|	
Mobile phase	ACN: Methanol: 0.1% OPA = 60:30:10
Column	Symmetry ODS (C_{18}) RP Column, 250 mm x 4.6 mm, 5μm
Column Temperature	Ambient
Detection Wavelength	235 nm
Flow rate	1.0 ml/ min.
Run time	06 min.
Temperature of Auto Sampler	Ambient
Diluent	Mobile Phase
Injection Volume	10μl
Type of Elution	Isocratic
Retention time	2.570 minutes

Fig-3: Chromatogram of Dasatinib in Optimized Condition

Observation: The selected and optimized mobile phase was ACN: Methanol: 0.1% OPA = 55:35:10% v/v/v and conditions optimized were flow rate (1.0 ml/minute), wavelength (235nm), Run time was 06 mins[9]. Here the peaks were separated and showed better resolution, theoretical plate count and symmetry. The proposed chromatographic conditions were found appropriate for the quantitative determination of the drug[10].

Method Validation

1. Accuracy:

Recovery Study:

To determine the accuracy[11] of the proposed method, recovery studies were carried out by adding different amounts (80%, 100%, and 120%) of pure drug of Dasatinib were taken and 3 replications of each has been injected to HPLC system. From that percentage recovery values were calculated from the linearity equation[12] y = 19423x + 5444.4. The results were shown in table-5.

Table 5: Readings of Accuracy

Conc. In ppm	Conc. Found	Peak Area		% Recovery
8	8.035	161523		100.437
8	8.153	163815		101.912
8	8.061	162023		100.762
			Avg.	101.037
			S.D	0.775
			%RSD	0.767046
Conc. In ppm	Conc. Found	Peak Area		% Recovery
10	9.930	198315		99.30
10	10.033	200320		100.33
10	10.044	200540		100.44
			Avg.	100.0233
			S. D	0.628835
			%RSD	0.628688
Conc. In ppm	Conc. Found	Peak Area		% Recovery
12	11.981	238151		99.841
12	12.066	239819		100.55
12	12.215	242712		101.791
			Avg.	100.7273
			S.D	0.987021
			%RSD	0.979894

2. Precision:

2.1. Repeatability

The precision[13] of each method was ascertained separately from the peak areas & retention times obtained by actual determination of six replicates of a fixed amount of drug. Dasatinib (API). The percent relative standard deviation[14] was calculated for Dasatinib are presented in the table-6.

Table 6: Readings of Repeatability

HPLC Injection Replicates of Dasatinib	Retention Time (Minutes)	Peak Area (AUC)
Replicate – 1	2.572	197236
Replicate – 2	2.570	197762
Replicate – 3	2.573	195969
Replicate – 4	2.570	194724
Replicate – 5	2.574	198327
Replicate – 6	2.573	198711
Average		197121.5
Standard Deviation		1515.213
% RSD		0.768667

Observation: The repeatability study[15] which was conducted on the solution having the concentration of about 10μg/ml for Dasatinib (n =6) showed a RSD of 0.768667% for Dasatinib. It was concluded that the analytical technique showed good repeatability.

2.2. Intermediate Precision/Ruggedness:

2.2.1. Intra-Day & Inter-Day:

The intra & inter day variation[16] of the method was carried out & the high values of mean assay & low values of standard deviation & % RSD (% RSD < 2%) within a day & day to day variations for Dasatinib revealed that the proposed method is precise.

Intra Day/Day-1/Analyst-1:

Table 7: Results of Intermediate Precision Analyst 1 for Dasatinib

S.No.	Peak Name	RT	Area (μV*sec)	USP Plate count	USP Tailing
1	Dasatinib	2.580	206587	3102	1.16
2	Dasatinib	2.597	206859	2986	1.18
3	Dasatinib	2.581	207854	3054	1.13
4	Dasatinib	2.573	208965	3154	1.14
5	Dasatinib	2.590	206547	3157	1.12
6	Dasatinib	2.572	209865	3268	1.18
Mean			207779.5		
Std. Dev.			1381.9336		
% RSD			0.665		

Inter Day/Day-2/Analyst-2:

Table 8: Results of Intermediate Precision Analyst 2 for Dasatinib

S.No.	Peak Name	RT	Area (µV*sec)	USP Plate count	USP Tailing
1	Dasatinib	2.580	215263	3215	1.17
2	Dasatinib	2.597	214235	3652	1.19
3	Dasatinib	2.581	213254	3496	1.15
4	Dasatinib	2.573	212367	3258	1.16
5	Dasatinib	2.590	213698	3365	1.17
6	Dasatinib	2.572	217456	3524	1.14
Mean			**214378.8**		
Std. Dev.			**1791.516**		
% RSD			**0.835678**		

Observation: Intraday and interday studies show that the mean RSD (%) was found to be within acceptance limit (≤2%), so it was concluded that there was no significant difference for the assay, which was tested within day and between days. Hence, method at selected wavelength[17] was found to be precise.

3. Linearity & Range:

The calibration curve[18] showed good linearity in the range of 6 – 14 µg/ml, for Dasatinib (API) with correlation coefficient (r^2) of 0.999 (Fig-4). A typical calibration curve has the regression equation of y = 19423x + 5444.4 for Dasatinib.
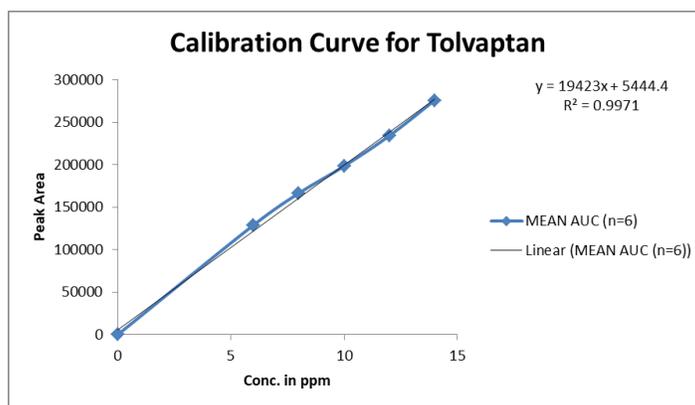

Fig 4: Calibration Curve of Dasatinib (API)
Table 9: Results of Linearity

CONC.(µg/ml)	MEAN AUC (n=6)
0ppm	0
6ppm	129013
8ppm	166523
10ppm	198315
12ppm	234151
14ppm	275819

Linearity Plot:

The plot of Concentration (x) versus the Average Peak Area (y) data of Dasatinib is a straight line.

$Y = mx + c$

Slope (m) = 19423

Intercept (c) = 5444.4

Correlation Coefficient (r) = 0.99

Validation Criteria: The response linearity is verified if the Correlation Coefficient[19] is 0.99 or greater.

Conclusion: Correlation Coefficient (r) is 0.99, and the intercept is 5444.4. These   values meet the validation criteria.

4. Specificity:

The system suitability for specificity[20] was carried out to determine whether there was any interference of any impurities in the retention time of the analytical peak.
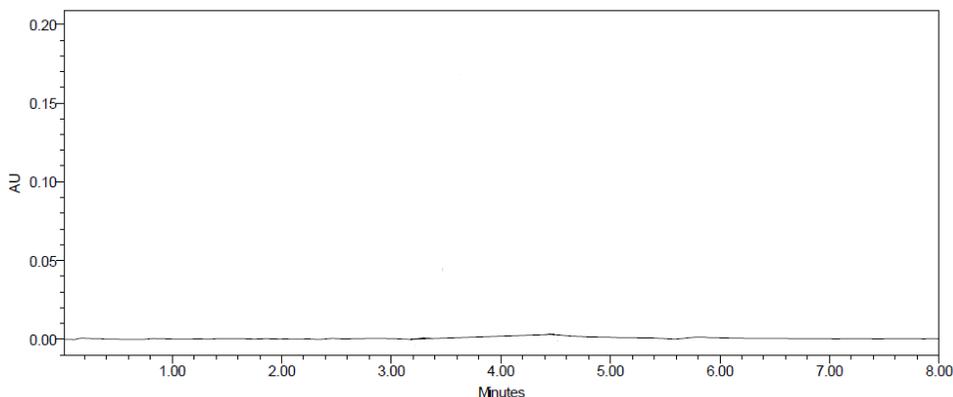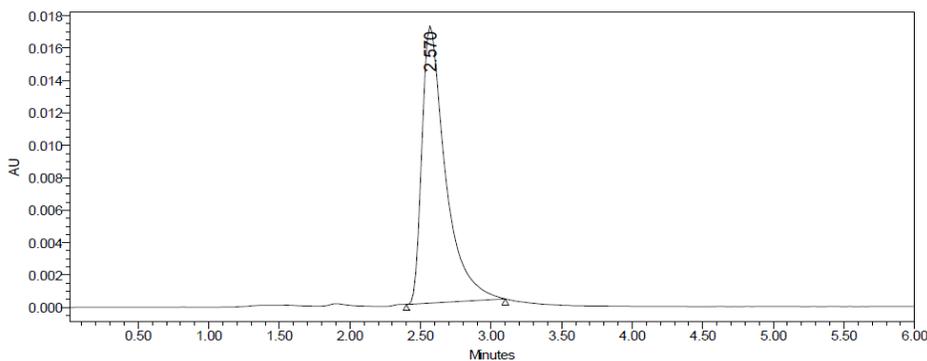

Fig-5: Chromatogram for Blank Solution

Fig-6: Chromatogram of Dasatinib Standard Solution

Observation: The study was performed by injecting blank and standard into the system. There was no interference of any peak in the blank with the retention time of the analytical peaks.

5. Method Robustness: Influence of small changes in chromatographic conditions such as change in flow rate (± 0.1ml/min), Wavelength of detection[21] (±2nm) & organic phase in mobile phase (±5%) studied to determine the robustness of the method are also in favour of (Table-10, % RSD < 2%) the developed RP-HPLC method[22] for the analysis of Dasatinib (API).

Table 10: Results for Robustness for Dasatinib

Parameter Used for Sample Analysis	Peak Area	Retention Time	Theoretical Plates	Tailing Factor
Actual Flow rate of 1.0 mL/min	203654	2.570	2915	1.16
Less Flow rate of 0.9 mL/min	265876	2.573	3652	1.19
More Flow rate of 1.1 mL/min	298653	2.631	3854	1.20
Less Organic Phase	315874	2.590	3945	1.17
More Organic Phase	326985	2.602	3487	1.19

6. LOD & LOQ:

LOD: The detection limit[23] of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value.

LOD = 3.3 × σ / s

Where

σ = Standard deviation of the response

S = Slope of the calibration curve

LOQ: The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined.

LOQ = 10×σ/S

Where

σ = Standard deviation[24] of the response

S = Slope of the calibration curve

Observation: The Minimum concentration level at which the analyte can be reliable detected (LOD) & quantified[25] (LOQ) were found to be 0.08 & 0.24µg/ml respectively.

7. System Suitability Parameter: System suitability[26-28] was carried out with six injections of solution of 100% concentration having 10µg/ml of Avapritinib in to the chromatographic system. Number of theoretical plates (N) obtained and calculated tailing factor (T) was reported in table-11.

Table 11: Data of System Suitability Parameter

S.No.	Parameter	Limit	Result
1	Asymmetry	T ≤ 2	Dasatinib=0.23
2	Theoretical plate	N > 2000	Dasatinib=2987
3	Tailing Factor	T<2	Dasatinib=1.17

8. Estimation of Dasatinib in Pharmaceutical Dosage Form

Twenty pharmaceutical dosage forms were taken and the I.P. strategy was taken after to decide the normal weight. Above measured tablets were at last powdered and triturated well. An amount of powder proportionate to 25 mg of medications were exchanged to 25 ml volumetric flagon, make and arrangement was sonicated for 15 minutes, there after volume was made up to 25 ml with same dissolvable. At that point 10 ml of the above arrangement was weakened to 100 ml with versatile stage. The arrangement was separated through a layer channel (0.45 µm) and sonicated to degas. The arrangement arranged was infused in five reproduces into the HPLC framework[29] and the perceptions were recorded.

A copy infusion of the standard arrangement was additionally infused into the HPLC framework and the peak regions were recorded. The information[30] is appeared in Table-12.

Assay % =

$$\frac{AT}{AS} \times \frac{WS}{DS} \times \frac{DT}{WT} \times \frac{P}{100} \times \text{Avg. Wt} = \text{mg/tab}$$

Where:

AT = Peak Area of medication acquired with test arrangement

AS = Peak Area of medication acquired with standard arrangement

WS = Weight of working standard taken in mg

WT = Weight of test taken in mg

DS = Dilution of Standard arrangement

DT = Dilution of test arrangement

P = Percentage virtue of working standard

Table-12: Recovery Data for Estimation of Dasatinib in Invista-100 Tablet

Brand Name of Dasatinib	Labelled amount of Drug (mg)	Mean Dasatinib, RP-HPLC, Validation, Accuracy, Precision, Robustness, ICH Guidelines. (± SD) amount (mg) found by the proposed method (n=6)	Assay % (± SD)
Invista-100 Tablet (Dr. Reddy's)	100mg	99.528 (± 0.368)	99.479 (± 0.269)

Result & Discussion: The amount of drug in Invista-100 Tablets was found to be 99.528 (± 0.368) mg/tab for Dasatinib & % assay[31] was 99.479%.

SUMMARY AND CONCLUSION

To develop a precise, linear, specific & suitable stability indicating RP-HPLC method for analysis of Dasatinib, different chromatographic conditions were applied & the results observed are presented in previous chapters. Isocratic elution is simple, requires only one pump & flat baseline separation for easy and reproducible results. So, it was preferred for the current study over gradient elution. In case of RP-HPLC various columns are available, but here Symmetry ODS (C_{18}) RP Column, 250 mm x 4.6 mm, 5µm Column was preferred because using this column peak shape, resolution and absorbance were good. Discovery wavelength was chosen in the wake of examining the standard arrangement of medication more than 200 to 400nm. From the U.V range of Dasatinib, it is apparent that a large portion of the HPLC works can be proficient in the wavelength scope of 210-300 nm helpfully. Further, a stream rate of 1.0 ml/min and an infusion volume of 10µl were observed to be the best investigation. The outcome demonstrates the created technique is, amazingly, one more reasonable strategy for measuring and dependability related debasement examines, which can help in the investigation of Dasatinib in various details.

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