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ORIGINAL ARTICLE **OPEN ACCESS**

Analytical Method Development and Validation of Enzalutamide in Bulk Drug by Using RP-HPLC

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ABSTRACT
A reproducible stability indicating Reverse Phase-HPLC method for the quantification of Enzalutamide in pharmaceuticals was developed and validated. Chromatography was done on Agilent 1260 series with SB-C-18, 100x4.6 mm, 2.7µm column with Mobile phase 0.1% Acetic acid in Water: Acetonitrile (45:55 v/v) and 1 ml/min as a flow rate. Enzalutamide was detected at 235 nm UV wavelengths maximum. This method established with linearity coefficient value of 0.99 and the percentage recovery was found to be 99.3%. This method was proven with LOD and LOQ findings of 0.002162 ng/ml and 0/006485 ng/ml respectively. The drug was degraded in alkaline, oxidative and water degradation conditions and the percentage degradation values were 89.36, 97.09 and 96.96 % respectively. There was less degradation of drug when exposed to acid and UV conditions. The developed technique will be useful in the routine analysis of Enzalutamide bulk drug.
Keywords: *Enzalutamide, prostate cancer, ICH, RP-HPLC, precision*

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INTRODUCTION

Enzalutamide was chemically designated as 4 -(3 -(4 –cyano-3- (trifluoromethyl) phenyl) -5, 5- dimethyl -4-oxo-2- thioxoimidazolidin-1-yl) -2-fluoro-N-methylbenzamide having molecular formula of $C_{21}H_{16}F_4N_4O_2S$ and molecular weight 464.44 g/mol. It is used in the treatment of prostate cancer, which act as competitive inhibitor of androgen receptor and helps in different stages of signalling pathway of cancer. Enzalutamide acts on androgens like dihydrotestosterone and testosterone. In also inhibits the binding of AR to DNA and AR-tocoactivator proteins. Enzalutamide is classified as second generation nonsteroidal antiandrogen as it has significantly pronounced antiandrogen activity than first generation drugs. It also acts as W741C-mutant AR antagonist. Its available marketed formulation is Xtandi capsules [1-6]. Literature survey of drug revealed that very few analytical procedures on LC-MS/MS, UV and RP-HPLC were reported for the analysis of enzalutamide [7-10]. The present research work is carried out to develop a sensitive, precise and accurate stability indicating method for the quantification of Enzalutamide in bulk drug.

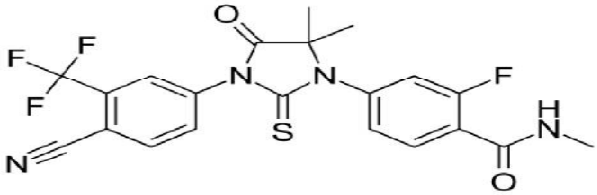


Fig 1. Structure of Enzalutamide

MATERIAL AND METHODS

Chemicals

Enzalutamide was received as a gift sample from Cipla Pharmaceuticals, India. All the HPLC grade chemicals were used for analysis.

Instrumentation

Agilent 1260 Series system was used which is equipped with quaternary pump, auto sampler unit, online degasser, column oven and PDA detector. All the data was processed and monitored by LC SOLUTION software.

Chromatographic conditions:

All optimized chromatographic conditions are described in Table 1

Table 1. Optimized HPLC chromatographic conditions of Enzalutamide

System	Agilent 1260 Series
Mobile Phase	0.1% Acetic acid in Water: Acetonitrile (45:55 v/v)
Flow rate	1 ml/min
Column	SB-C-18, 100 x 4.6 mm, 2.7 um, Agilent
Oven Temperature	40 degree Celsius
Wavelength of detection	235 nm
Back Pressure Observed	133-134 bar
Total run time	6 min
Average retention time	2.91 min

Mobile Phase preparation

It was processed by mixing HPLC grade acetonitrile, acetic acid and water. The resultant mobile phase sonicated for 10 to 15 mins for degasification and filtered through 0.45 micron filter paper [11-12].

Protocol for Standard Solution

Accurately weighed about 10 mg of Enzalutamide was transferred to a 10 ml volumetric flask and drug was dissolved in methanol completely, then the final volume was made up to 100 ml using methanol. Chromatogram was obtained as per optimized chromatographic conditions (table 1)

Method validation

The developed and optimized technique was validated for linearity, accuracy, solution stability, limits of detection (LOD), limits of quantitation (LOQ), intra and intraday precision compliance with ICH validation parameters [13-18].

Linearity & range

Linearity was determined by preparing different concentrations of drug [19]. Stock solution of concentration 1000µg/ml was diluted to get the different concentrations 10 µg/ml, 20µg/ml, 40µg/ml, 60 µg/ml, 80 µg/ml and 100 µg/ml. Concentration of 20µl each was then injected in the column and the chromatograms were obtained. A graph of Area under curve vs. concentration was then plotted to obtain calibration curve. Equation, slope, intercept and regression coefficient (R^2) were determined from obtained calibration curve.

Accuracy

% recovery was determined to find out method accuracy. In a preweighed drug solution, a known amount of drug was added & chromatogram was recorded as per the optimized chromatographic conditions.

Solution stability

Stability of solution was determined by analyzing the standard drug solution after storage for 24 hrs at laboratory conditions.

Precision

Precision represents the measurement of the degree of reproducibility of test results for the developed analytical method. It is of two types, repeatability (intra-day precision) and intermediate precision (inter-day precision). The precision of the method was evaluated for 12µg/ml, 50µg/ml, 95µg/ml. For intra-day precision, sets of three replicates of above concentration were analyzed on the same day and for inter-day precision, sets of same replicates were analyzed on multiple days [20-22].

Limit of Detection (LOD) [23-24]

The limit of detection (LOD) is the lowest concentration that can be detected but not appreciably quantified. LOD is calculated from the formula:

$LOD = 3.3\sigma/s$

Where, σ = standard deviation of the response

S = slope of the calibration curve.

Limit of Quantification (LOQ) [23-24]

It is lowest concentration of a compound that is practicable to be determined by means of a given analytical procedure. LOQ is calculated from formula:

$LOQ = 10\sigma/s$

Where,

σ = standard deviation of the response

S = slope of calibration curve

Forced degradation studies

Forced degradation studies were carried out with acid, alkaline, oxidative, water and ultra violet (UV) degradations on drug. The drug sample was processed by exposing to above stress conditions and the

peak purity was determined from the obtained chromatograms, which indicates that the technique was effectively separated the degrade compounds from the standard [25-28].

Acid degradation

0.125 mg of Enzalutamide pure drug was transferred in to a 10 ml volumetric flask. Drug was dissolved in 5 ml of 0.1 N Hydrochloric acids and solution was exposed to 80°C temperature in a hot water bath for 2 hours. The resulting solution was filtered through 0.22 micrometer filter paper and analyzed by optimized chromatographic conditions [16, 29-30].

Alkali degradation

0.125 mg of Enzalutamide pure drug was transferred in to a 10 ml volumetric flask. Drug was dissolved in 5 ml of 0.1 N sodium hydroxide and solution was exposed to 80°C temperature in a hot water bath for 2 hours. The resulting solution was filtered through 0.22 micrometer filter paper and analyzed by optimized chromatographic conditions [16, 29-30].

Oxidative degradation

0.125 mg of Enzalutamide pure drug was transferred in to a 10 ml volumetric flask. Drug was dissolved in 5 ml of 10% H2O2 and solution was exposed to 80°C temperature in a hot water bath for 2 hours. The resulting solution was filtered through 0.22 micrometer filter paper and analyzed by optimized chromatographic conditions [16, 29-30].

Water degradation

0.125 mg of Enzalutamide pure drug was transferred in to a 10 ml volumetric flask. Drug was dissolved in 5 ml of distilled water and solution was exposed to 80°C temperature in a hot water bath for 2 hours. The resulting solution was filtered through 0.22 micrometer filter paper and analyzed by optimized chromatographic conditions [31].

UV-degradation

2 mg of Enzalutamide was accurately weighed and placed in clean petridish. Then the petridish was kept under a UV chamber for 2 hours by maintaining 30 cm distance. The drug was exposed to UV. After 2hrs, the UV lamp was switched off. 20µg/ml concentration was processed by serial dilution with diluent. The resulting solution was filtered through 0.22 micrometer filter paper and analyzed by optimized chromatographic conditions [32].

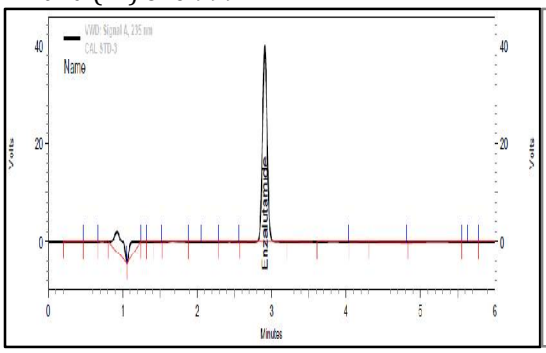
RESULT AND DISCUSSION

A typical chromatogram of Enzalutamide

Based on the results obtained from screening studies, C18 column and 0.1% Acetic acid in Water: Acetonitrile (45:55 v/v) was selected for the analysis of Enzalutamide. The applied chromatographic conditions permitted a good separation of Enzalutamide with a short retention time of 2.91 min.

Linearity & range:

The proposed method was found linear in the range of 10µg/ml-100µg/ml with an equation y = 73769x-32114 and (R^2) of 0.999.



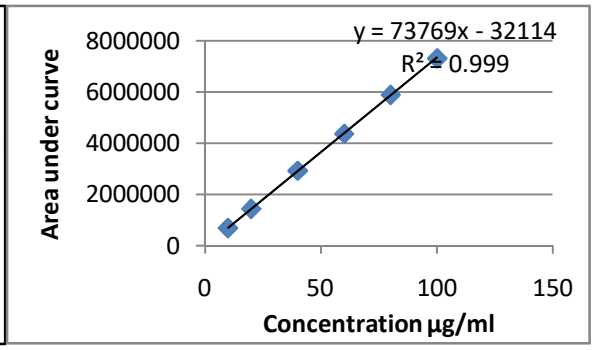


Fig.2.A typical chromatogram of Enzalutamide **Fig.3.Calibration curve of Enzalutamide**

Precision:

The % R.S.D values for intra and inter day precision studies were found less than 2 %, which indicates that the method was found to be precise.

Table 2. Intraday and Interday precision of Enzalutamide

Conc. (µg/ml)	Mean conc.		SD.		SEm		Co-efficient of Variation		% Co-efficient of Variation	
	Intra day	Inter day	Intra day	Inter day	Intra day	Inter day	Intra day	Inter day	Intra day	Inter day
12	11.30333	11.13667	0.168028	0.105987	0.097011	0.061192	0.014865	0.009517	1.4865	1.4865
50	48.0696	48.0696	0.407216	0.237286	0.235106	0.136997	0.008471	0.004936	0.8471	0.8471
95	90.39603	90.39603	0.317262	0.272149	0.183171	0.157126	0.00351	0.003011	0.351	0.351

Accuracy

Accuracy should be determined across the specified range of 98-102 %. The % accuracy was found within the specified limits.

LOD and LOQ

The determination of LOD and LOQ was based on the standard deviations of the responses and slopes of obtained calibration curves (n = 3) as per ICH guidelines Q2 (R1). The LOD and LOQ values were 0.002162 ng/ml and 0.006485 ng/ml respectively.

Robustness

The results indicate no significant changes occur with changing the chromatographic conditions such as flow rate and wavelength. This indicates that the developed method was found to be robust.

Ruggedness

The results obtained were not affected and was same as the original results. This confirms ruggedness of developed method.

System suitability study

System suitability tests are having importance in liquid chromatographic methods. Retention time, number of theoretical plates and peak area were calculated for standard solutions. The values for retention time, number of theoretical plates and peak area obtained from system suitability study were found to be 2.91 min, 12470 and 720022 respectively. The data was within acceptable limits.

Forced degradation studies

The Enzalutamide was subjected to stress conditions in various ways to observe the rate and extent of degradation that is likely to occur in the course of storage or after administration of drug into the body. This is one type of accelerated stability studies that helps us determining the degradation of the drug that is likely to occur after long time storage or within a very short time when compared with real time or long term stability studies. The various degradation pathways studied are acid hydrolysis, basic hydrolysis, UV degradation, water and oxidative degradation. Enzalutamide was stable when exposed to 0.1N HCL and UV light which is indicated by less degradation.

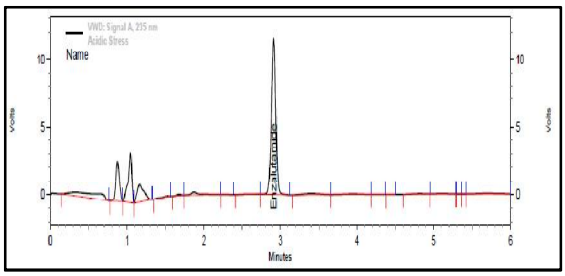


Fig.4. HCL mediated degradation

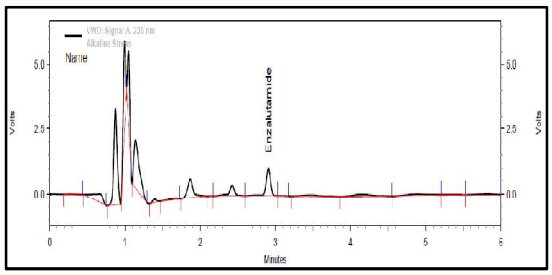


Fig.5.NaOH mediated degradation

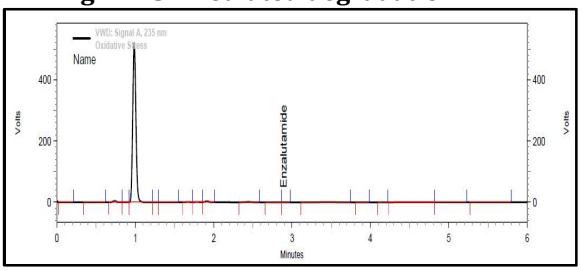


Fig.6.Hydrogen peroxide mediated degradation

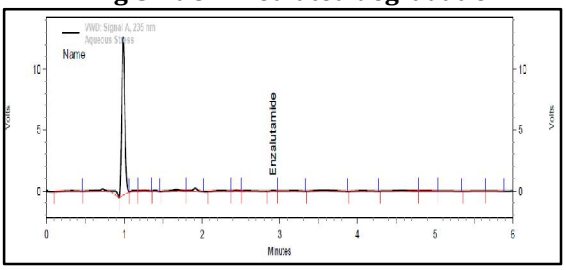


Fig.7.Water mediated degradation

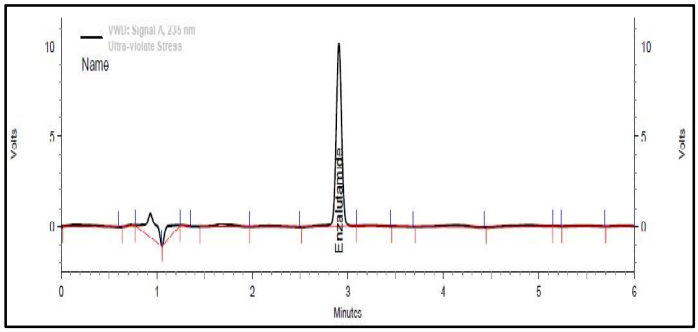


Fig.8.Degradation under UV light

CONCLUSION

RP-HPLC method was developed for Enzalutamide bulk drug. The chromatographic condition for optimized method was found to consisting of column SB-C-18, 100x4.6 mm, 2.7µm. Mobile phase was 0.1% Acetic acid in Water: Acetonitrile (45:55 v/v). The retention time were found to be 2.91 min. The proposed methods were found to comply with ICH guidelines. This method can be further employed in future for the routine determination of Enzal in bulk drug.

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Conflict of Interest

Authors do not have any conflict of interest.

REFERENCES

1. Gibbons, J. A., Ouatas, T., Krauwinkel, W., Ohtsu, Y., van der Walt, J. S., Beddo, V., ... & Mordenti, J. (2015). Clinical pharmacokinetic studies of enzalutamide. *Clinical pharmacokinetics*, *54*, 1043-1055.
2. Kim, T. H., Jeong, J. W., Song, J. H., Lee, K. R., Ahn, S., Ahn, S. H., ... & Koo, T. S. (2015). Pharmacokinetics of enzalutamide, an anti-prostate cancer drug, in rats. *Archives of pharmacal research*, *38*, 2076-2082.
3. Benoist, G. E., Hendriks, R. J., Mulders, P. F., Gerritsen, W. R., Somford, D. M., Schalken, J. A., ... & van Erp, N. P. (2016). Pharmacokinetic aspects of the two novel oral drugs used for metastatic castration-resistant prostate cancer: abiraterone acetate and enzalutamide. *Clinical pharmacokinetics*, *55*, 1369-1380.
4. Simondsen, K., & Kolesar, J. (2013). New treatment options for castration-resistant prostate cancer. *American journal of health-system pharmacy*, *70*(10), 856-865.
5. Scher, H. I., Fizazi, K., Saad, F., Taplin, M. E., Sternberg, C. N., Miller, K., ... & de Bono, J. S. (2012). Increased survival with enzalutamide in prostate cancer after chemotherapy. *New England Journal of Medicine*, *367*(13), 1187-1197.
6. Antonarakis, E. S. (2013). Enzalutamide: the emperor of all anti-androgens. *Translational andrology and urology*, *2*(2), 119.
7. Saini, N. K., Sulochana, S. P., Zainuddin, M., & Mullangi, R. (2018). Development and validation of a novel method for simultaneous quantification of enzalutamide, darolutamide and their active metabolites in mice dried blood spots using LC-MS/MS: Application to pharmacokinetic study in mice. *ADMET and DMPK*, *6*(3), 242-257.
8. Prajapati, D. J., Chhalotiya, U. K., Prajapati, M. D., Patel, J. U., & Desai, J. V. (2019). Quantification of Newly Discovered Anti-Cancer Drug Enzalutamide in Bulk and Synthetic Mixture by Stability Indicating TLC Method. *Current Drug Discovery Technologies*, *16*(1), 104-112.
9. Benoist, M., van der Meulen, E., Van Oort, I., Schalken, J., Gerritsen, W., Burger, D., & van Erp, N. P. (2016). Development and validation of a bioanalytical assay on LC/MS/MS to quantify enzalutamide and N-desmethylenzalutamide in human plasma.
10. Puszkiel, A., Plé, A., Huillard, O., Noé, G., Thibault, C., Oudard, S., ... & Blanchet, B. (2017). A simple HPLC-UV method for quantification of enzalutamide and its active metabolite N-desmethyl enzalutamide in patients with metastatic castration-resistant prostate cancer. *Journal of Chromatography B*, *1058*, 102-107.
11. Patil, N., Balaji, O., Udupa, K. S., & Rao, K. (2017). Bortezomib induced subconjunctival hemorrhage. *Asian Journal of Pharmaceutical and Clinical Research*, *10*(4), 10-11.
12. Charde, M. S., Welankiwar, A. S., & Chakole, R. D. (2014). Development of validated RP-HPLC method for the simultaneous estimation of atenolol and chlorthalidone in combine tablet dosage form. *International Journal of Advanced Pharmaceutical Sciences*, *3*, 6-18.
13. Decleer, M., Rajkovic, A., Sas, B., Madder, A., & De Saeger, S. (2016). Development and validation of ultra-high-performance liquid chromatography–tandem mass spectrometry methods for the simultaneous determination of beauvericin, enniatins (A, A1, B, B1) and cereulide in maize, wheat, pasta and rice. *Journal of Chromatography A*, *1472*, 35-43.
14. Madhavi, S., & Prameela Rani, A. (2018). Simultaneous reverse phase ultraperformance liquid chromatography method development and validation for estimation of Grazoprevir and Elbasvir. *Asian J Pharm ClinRes*, *11*, 100.

15. ICH, I. (2005, November). Q2 (R1): Validation of analytical procedures: text and methodology. In *International conference on harmonization, Geneva* (pp. 1-13).
16. ICH, M. (1996, March). Q2B validation of analytical procedures: methodology. In *Proceedings of the International Conference on Harmonization Expert Working Group, Geneva, Switzerland* (Vol. 6).
17. Prashanthi, Y., Rao, T. N., & Srinivas, Y. (2018). Method Development and Validation of Alectinib Drug by RP-HPLC in Bulk and Pharmaceutical Dosage Form. *Asian Journal of Pharmaceutical Analysis*, *8*(4), 186-190.
18. Sudha, T., & Mathew, B. E. (2012). Development and validation of RP-HPLC method of analysis for the quantitative estimation of Darifenacin in tablet dosage forms. *Research Journal of Pharmacy and Technology*, *5*(4), 518-522.
19. Gupta, S. K., Kumar, B., & Sharma, P. K. (2013). Development and validation of a Spectrophometric method for estimation of Triamcinolone in solid dosage form. *Asian Journal of Pharmaceutical Analysis*, *3*(2), 42-43.
20. Manchanda, S., Sahoo, P. K., & Majumdar, D. K. (2016). RP-HPLC method development and validation for the estimation of Acetazolamide in bulk drug and formulations with forced degradation studies. *Pharm Lett*, *8*, 338-47.
21. Rajamanickam, V., Santhosam, D., Sridharan, D., & Thenmozhi, A. (2010). Simultaneous determination of risperidone and Trihexhyphenidyl hydrochloride from bulk and tablet dosage form by RP-HPLC. *Asian Journal of Research in Chemistry*, *3*(3), 549-551.
22. Babu, G., Perumal, P., Manickam, V. B., Srikakolapu, S. R., Gotteti, S. D., & Thirumurthy, L. S. (2010). Simultaneous Determination of Strychnine and Brucine in Herbal Formulation by RP-HPLC. *Asian Journal of Research in Chemistry*, *3*(3), 720-723.
23. Rajamanickam, V., Santhosam, D., Sridharan, D., & Thenmozhi, A. (2010). Simultaneous determination of risperidone and Trihexhyphenidyl hydrochloride from bulk and tablet dosage form by RP-HPLC. *Asian Journal of Research in Chemistry*, *3*(3), 549-551.
24. Ganesan, B., Perumal, P., Manickam, V., Srikakolapu, S. R., Gotteti, S. D., & Thirumurthy, L. S. (2010). Simultaneous determination of strychnine and brucine in herbal formulation by UV derivative spectrophotometry. *Int. J. Pharm. Tech. Res*, *2*, 1528-1532.
25. Patil, S. D., Varpe, P., Chaure, S., Bhalerao, S. B., & Kshirsagar, S. (2017). Development and Validation of Stability Indicating RP-HPLC Method for Piracetam. *Asian Journal of Research in Pharmaceutical Sciences*, *7*(4), 215-221.
26. Patil, S. D., Amurutkar, S. V., & Upasani, C. D. (2016). Development and validation of stability indicating RP-HPLC method for empagliflozin. *Asian Journal of Pharmaceutical Analysis*, *6*(4), 201-206.
27. Sinha, P. K., Jeswani, R. M., Topgi, K. S., & Damle, M. C. (2010). Stability Indicating RP-HPLC Method for Determination of Potassium Clavulanate in the presence of its Degradation Products. *Research Journal of Pharmacy and Technology*, *3*(1), 141-145.
28. Ingole, L. R. (2019). Development of Stability Indicating RP-HPLC Method for Estimation of Clemastine Fumarate and its Pharmaceutical Dosage Form. *Research Journal of Pharmaceutical Dosage Forms and Technology*, *11*(2), 87-94.
29. International Conference on Harmonisation (ICH), 2005. Harmonised Tripartite Guideline: Validation of Analytical Procedures: Methodology (Q2B), 2005.
30. Pinaz, A. K., & Muralikrishna, K. S. (2013). Method development and acid degradation study of rivaroxaban by RP-HPLC in bulk. *Asian Journal of Pharmaceutical Analysis*, *3*(2), 62-65.
31. Jahan, M. S., Islam, M. J., Begum, R., Kayesh, R., & Rahman, A. (2014). A study of method development, validation, and forced degradation for simultaneous quantification of paracetamol and ibuprofen in pharmaceutical dosage form by RP-HPLC method. *AnAlyticAl chemistry insights*, *9*, 75.
32. Mule, K. L. (2017). Rapid analytical method for assay determination for prochlorperazine edisylate drug substances by ultra-performance liquid chromatography. *Int J Curr Pharm Res*, *9*(4), 118-122.

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