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Comparison of FT-NIR and HPLC for Green Approach to Assay Cefixime in Its Pharmaceutical Capsules

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Near-infrared spectroscopy (NIRS) has become an analytical technique of great interest for the pharmaceutical industry, particularly as a greener analytical technique. In this paper a calibration model for cefixime in the capsules pharmaceutical dosage form was built by utilizing chemometric processing based mainly on a partial least square regression fit to assay the material using NIR technique. The results obtained by NIR spectroscopy were compared with the compendial HPLC method for cefixime capsules produced by USP. The present study showed that cefixime capsules can be individually analyzed by NIR with high accuracy. This proposed technique realizes a lot of green analytical aspects in developing eco-friendly analytical method

Keywords: cefixime capsules, FT-NIR transmission, GAC (green analytical chemistry), HPLC assay, PAT (process analytical technology), validation; PLS model

INTRODUCTION

The trend of sustainable development requires chemistry to be "clean" or "green." In the 1990s, therefore, the concept of "Green Chemistry" was proposed, together with the "Twelve Principles of Green Chemistry." Presently, spectroscopic methods dominate the area of green analytical chemistry [1]. The pursuit in the field of green chemistry is growing dramatically and is becoming a grand challenge for chemists to develop new products, processes and services that achieve the necessary social, economical and environmental objectives due to an increased cognizance of environmental safety, checking environmental pollution, sustainable industrial ecology and cleaner production technologies worldwide [1]. At the same time the trend of process analytical technology (PAT) is highly encouraged by the FDA. PAT is a collaboration effort with industry to facilitate the introduction of new and efficient manufacturing technologies [2]. PAT are systems for design, analysis,

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and control of manufacturing processes, based on timely measurements of critical to quality (CTQs) attributes of raw and in-process materials and products, to assure high quality of products at the completion of manufacturing processes [3]. PAT includes scientifically based process design that identifies key measurements of product quality and the critical process variables that affect them, appropriate measurement devices, statistical information technology tools, and feedback process control strategies that work together to ensure production of final products with the desired quality. Several spectroscopy techniques are used for the application of PAT in on-line, in-line and off-line monitoring of the pharmaceutical process. For several years, near-infrared spectroscopy has become an analytical technique of great interest for the pharmaceutical industry because it is a rapid, non-destructive technique also it requires none or minimal sample pretreatment and do not utilize toxic solvent or reagents which make it one of the most favorable spectroscopic techniques according to the GAC aspects.

The NIR region spans the wavelength range 12,500–4000 cm^{-1}. In this region, absorption bands correspond mainly to overtones and combinations of fundamental vibrations [4]. In the pharmaceutical sector, several qualitative and quantitative applications of NIR spectroscopy have been described during manufacturing steps. In the beginning of manufacturing process, NIR can be used for the identification of active substances and excipients [5–7]. NIR were known as an interesting tool for the quantitative analysis of raw materials and monitoring of the blending steps during manufacturing process [8] specially assuring the homogeneity of the powder or granules mix is a crucial step during manufacturing of the pharmaceuticals. In practice the relationship between concentration and absorbance is empirically determined by calibration. In the first step, spectra of substances with known composition are recorded. Then, these acquired spectra and the data available from a reference analysis are used to determine a calibration function. In the second step, spectra of substances with unknown composition are measured and then used to determine the properties of interest by means of the calibration function [9, 10].

Several methods for evaluation of cefixime have been reported such as spectrophotometric in dosage form [11, 12], fluorimetric in raw material and dosage forms [13], voltammetric in dosage forms and biological fluids [14, 15], high-performance liquid chromatographic in dosage forms and biological fluids [16–20], and high-performance thin-layer chromatographic in dosage form [21] methods. However, using NIR technique is a quite new approach considering the GAC aspects. The aim of this study is to show the agreement between the NIR as a greener technique and the conventional HPLC – UV detection method, official in USP.

EXPERIMENTAL

Materials and methods

Materials

All materials were kindly supplied by SIGMA pharmaceuticals Corp., Egypt. The commercial samples of Ximacef capsules (400 mg cefixime) were used and a placebo contains the same raw materials used in the production process, was used including avicel PH102, croscarmellose, magnesium stearate and purified talc. The placebo was used to make serials of dilutions for establishing the calibration model. All materials are of pharmaceutical grade and all chemical and reagents have been used in the HPLC method are analytical reagent of HPLC grade.

Analytical procedures

NIR spectroscopy

FT-NIR Spectrometer, MPA Flexible from Bruker Optics (Germany) was used. It is equipped with 2 fiber optic probes one for solid and the other for liquid samples, each one has 2 m fiber optic cable. The spectrometer has external transmission unit and sample wheel for direct measurement of dosage units which were used in this study. The spectrometer is equipped with a fast, PC-based data system with OPUS/IR FT-IR spectroscopy software. OPUS IDENT is a software package designed to identify substances by their NIR spectra while OPUS Quant is designed for the quantitative analysis. For this purpose, OPUS QUANT was used with a partial least square (PLS) fit method.

Constructing the PLS model

At first step a PLS regression model was built using calibration samples. In PLS, the calibration involves correlating the data in the spectral matrix *X* with the data in the concentration matrix *Y*. This means that the factoring of the spectral data is more suited for concentration prediction. The obtained model was chemometrically validated by leave-one-out cross validation. The final PLS model was described by a selected spectral region, spectra pretreatment and a number of PLS factors. To build the model, 42 different concentrations of capsules were prepared ranged from 0 % to 100% of the labeled amount and 5 samples were measured per concentration. To obtain these different concentrations, raw materials and placebo are used and samples were prepared on an experimental scale. Each spectrum was the average of 32 scans and the spectrophotometer was operated at a resolution of 8 cm^{-1}.

Spectral data pretreatments

Calibration models were developed using full cross validations. The baseline can drift and maximum absorbance may change. Spectral pretreatments correct these interferences [22,23]. In this study, reducing of baseline drift and enhancing spectral information has been achieved through chemometric processing include first derivative, second derivative, vector normalization, straight line subtraction and constant offset elimination. The absorbance of the Cefixime capsules samples was measured and NIR spectra were saved. Measurements were performed by the external transmission unit working on diffuse reflection mode and sample wheel. Before measurement a measurement for the background must be taken and the detector signal shall be checked. In developing method, the measurement conditions shall be determined and saved to be recalled in each measurement time to avoid result variation and ensure high accuracy of the developed analytical procedure. Samples were measured into samples cavities of the equipment tray with keeping minimum illumination in the measurement place by using samples cover to ensure that there was no stray light during measurement. The measurement time for each sample was about 10 seconds per scan and the instrument was operated at a resolution of 8 cm^{-1}. In first trials 3 scans for each sample were taken and a mean spectrum for them was calculated but it was found that no difference between the mean spectrum and the single one that is due to the complete homogeneity of the measured sample.

Reference method

According to the USP, the official HPLC method utilizing C_{18} (125×4.6 mm, 4 µm) separation column at 40°C and mobile phase composed of 0.01 M tetrabutyl-amonium hydroxide solution, pH was adjusted with phosphoric acid to 6.5 ± 0.5 and acetonitrile (3 : 1). The flow rate to be adjusted at 1 mL/min. and the detection wavelength was set at 254 nm[24].

RESULTS AND DISCUSSIONS

Methods validation

Figure 1 shows the raw spectra obtained with the calibration samples. From these spectra, five regions were selected, automatically by the Quant program; it was between 6000 and 5500 cm^{-1}. All samples were also analyzed by the HPLC official method; Figure 2 shows the resulting chromatogram. As the HPLC reference method is official in the USP, the researcher has performed verification study include the items of linearity, sensitivity, accuracy and precision for the compendial method while full cross validation has been performed for the proposed NIR method and the validation statistics obtained for the calibration models are recorded in Table 1.

Linearity for the reference method

The linearity of calibration curves (peak area vs. concentration) for cefixime in pure solutions as well as in the drug-matrix solutions were checked over the concentration ranges of 5, 10, 15, 20, 25, 30, 50 and 100 µg/mL, each concentration level was injected 3 times (n=3) and the average peak area was calculated. The resulting curve was found to be linear with correlation coefficients of better than 0.999 in most cases; the limits of detection LOD and limits of quantitation LOQ, were calculated for the calibration curve of cefixime as three and ten times of the noise level for LOD and LOQ, respectively [25]. Table 2 lists the linearity parameters of the calibration curves for cefixime in pure drug and drug-matrix preparations.

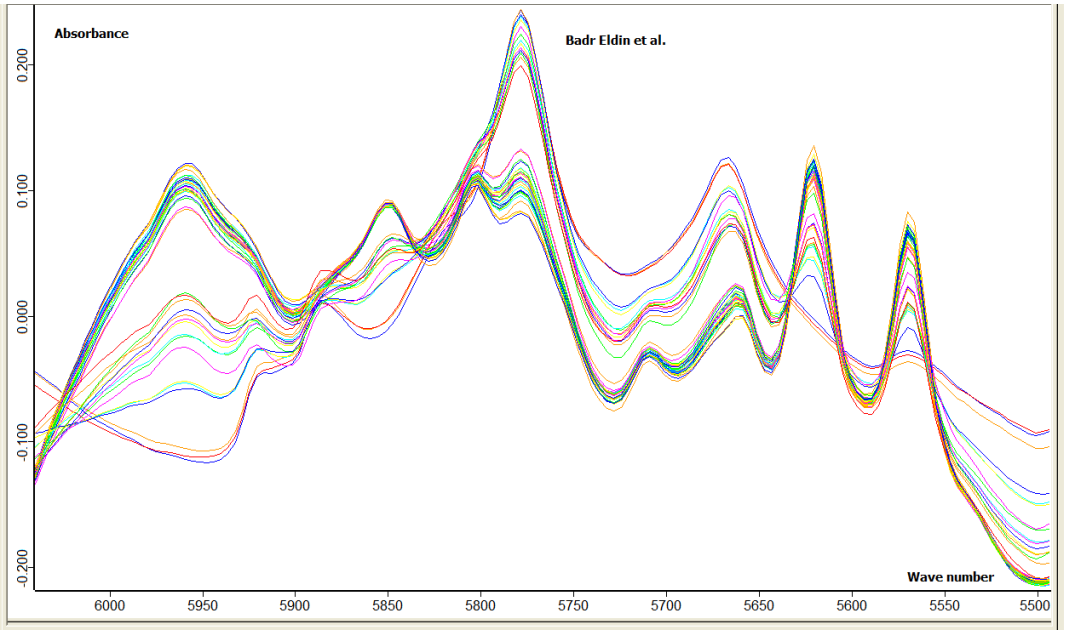

Figure 1. The FT-NIR spectra for cefixime capsules of different concentrations described in USP.

Table 1: Full cross-validation statistics obtained with calibration models for cefixime in measured in cefixime capsules by NIR spectroscopy

True	Prediction	F Value**	F Prob*	Difference
0	-1.681	1.78	0.811	1.68
0	-1.301	1.05	0.688	1.3
0	3.458	8.7	0.995	-3.46
97.27	97.09	0.0189	0.109	0.177
96.75	95.33	1.26	0.732	1.42
96.51	97.07	0.19	0.335	-0.559
91.32	91.81	0.148	0.297	-0.493
90.27	91.79	1.44	0.763	-1.52
89.38	88.21	0.848	0.638	1.17
93.09	92.66	0.111	0.259	0.427
89.42	88.46	0.56	0.542	0.956
91.78	91.13	0.259	0.387	0.652
90.11	89.13	0.588	0.553	0.979
89.99	89.62	0.0819	0.224	0.367
88.45	87.39	0.697	0.592	1.06
88.85	87.62	0.932	0.66	1.23
84	83.97	0.000588	0.0192	0.0312
83.6	82.71	0.486	0.511	0.891
86.22	86.17	0.00134	0.0291	0.0471
83.01	82.93	0.00376	0.0486	0.0789
85.09	84.67	0.105	0.252	0.415
81.94	82.18	0.035	0.148	-0.24
83.08	83.38	0.053	0.181	-0.296
80.95	81.2	0.0385	0.155	-0.252
79.39	79.98	0.212	0.352	-0.59
76.8	78.68	2.25	0.859	-1.88
77.71	78.5	0.38	0.459	-0.789
76.94	77.89	0.55	0.538	-0.948
24.66	24.55	0.00697	0.0662	0.107
23.82	23	0.408	0.474	0.817
18.56	18.25	0.0578	0.189	0.309
36.59	34.2	3.75	0.941	2.39
33.21	32.56	0.26	0.387	0.653
33.32	32.05	1.01	0.678	1.27
34.17	34.75	0.201	0.344	-0.575
38.15	37.97	0.0193	0.11	0.179
46.06	44.87	0.877	0.646	1.19
43.74	44.7	0.568	0.545	-0.963
42.36	43.47	0.758	0.611	-1.11
42.17	43.37	0.885	0.648	-1.2
38.52	42.74	14.4	1	-4.22
100.2	100.2	0.000589	0.00609	-0.00987
99.97	101.6	1.76	0.809	-1.67
99.24	100.6	1.2	0.72	-1.39

** F Value: reducing such value indicates that the spectra are efficiently represented by the PLS vectors*
***F Prob: indicates the probability that a standard is a spectral outlier.*

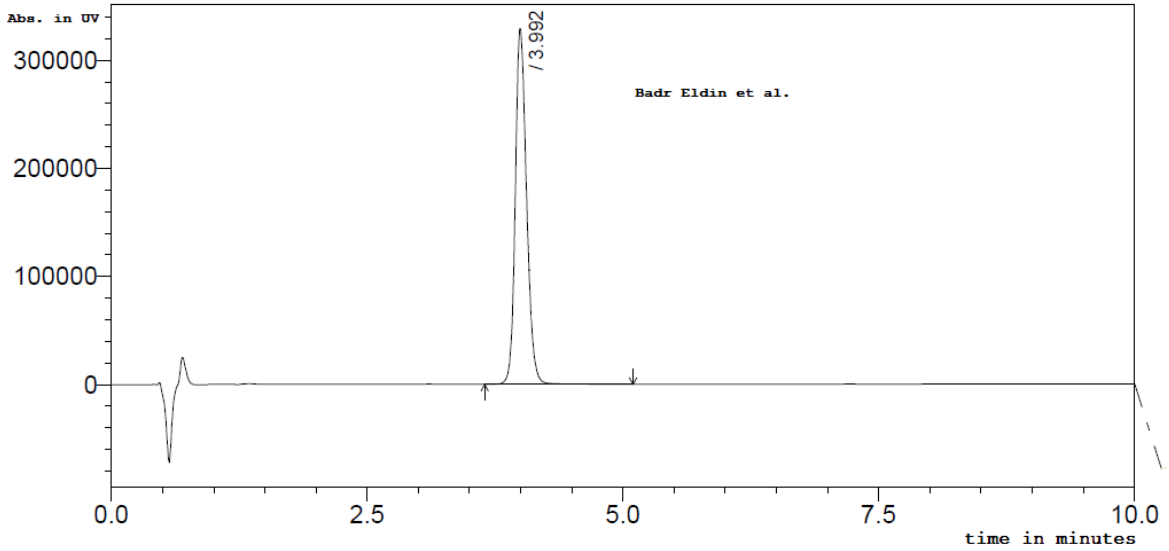

Figure 2. The resulting chromatogram for cefixime (100 mcg/mL) under typical chromatographic conditions

Table 2. Linearity of calibration curve for cefixime in standard preparations and in drug-matrix preparation. Number of points in the regression line is 6 for each case.

Item	Calibration range (mcg/mL)	Correlation coefficient	Slope	Slope 95% confidence interval for the slope[a]	intercept	Slope 95% confidence interval for the intercept[a]	LOQ (mcg/mL)	LOD (mcg/mL)
cefixime in standard preparation	5 - 50	0.9999	0.703	± 0.0239	0.347	± 2.331	5.75	1.05
cefixime in drug-matrix preparation	5 - 50	0.9999	0.609	± 0.0255	1.239	± 3.087	9.55	2.35

[a]Confidence intervals of the slope and the intercept = (S.D of the slope or intercept x *t*), the value of *t* at 3 degree of freedom and 95% confidence level is 3.25

Sensitivity and accuracy for the reference method

The accuracy of an analytical method is the closeness of test results obtained by that method to the true value [25]. The accuracy of the method was tested by analyzing different samples of cefixime at various concentration levels ranged from 5 to 50 µg/mL in either pure solutions or in solutions comprising the drug-matrix used in capsules formulation, each concentration level was injected 3 times (n=3) and the average peak area was calculated. The results were expressed as percent recoveries of the cefixime in the samples. Table 3 shows that the overall percent recoveries of cefixime in pure and drug-matrix solutions were 99.69 % and 100.37% with relative standard deviation (RSD) lower than 2% in all cases.

Table 3. Accuracy of the proposed HPLC method for the determination of cefixime in standard or drug matrix solution.

Quantity added in mcg/mL of cefixime	Standard solutions		Drug – matrix solutions	
	Quantity found in mcg/mL	Recovery (%)	Quantity found in mcg/mL	Recovery (%)
5.025	5.011	99.72	5.031	100.12
6.7	6.66	99.40	6.712	100.18
10.05	9.97	99.20	10.09	100.39
20.1	20.21	100.55	20.3	100.99
50.25	50.03	99.56	50.34	100.18
Calculations:	Average	99.69	Average	100.37
	% R.S.D	0.463	% R.S.D	0.363

Precision for the reference method

According to and ICH [26] guidelines, the precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. Precision may be considered at three levels: repeatability, intermediate precision and reproducibility.

Analysis repeatability

It was evaluated by carrying out the analysis of the six homogenous solutions of same test sample ranged from 30 – 50 mcg/mL. The determinations were carried out one after the other under conditions as similar as possible. The relative standard deviation was calculated from the results of the obtained observations (Table 4).

Table4: Repeatability and intermediate precision for the reference HPLC method

Exp. No.	Repeatability on Day 1		Repeatability on Day 2 (intermediate precision)	
	cefixime in standard preparation	cefixime in drug-matrix preparation	cefixime in standard preparation	cefixime in drug-matrix preparation
1	101.1%	100.23%	100.41%	100.35%
2	99.93%	100.16%	100.23%	100.26%
3	99.96%	100.52%	100.36%	100.91%
4	99.97%	99.54%	100.71%	100.51%
5	99.79%	99.94%	100.37%	100.46%
6	100.14%	100.94%	101.11%	100.62%
Mean	100.15%	100.22 %	100.53%	100.52%
SD	0.48	0.480	0.325	0.229
RSD	0.479%	0.479%	0.323%	0.228%

Intermediate Precision

The intermediate precision of the method was checked by determining precision on a different day using the same number of samples and same concentration range as in repeatability. The relative standard deviation was calculated from the results of the obtained observations. In all cases the RSD was lower than 2 (Table 4).

Predictability of the proposed NIR method

The coefficient of correlation ($r2$) and root mean square error of cross-validation (RMSECV) are essential tools to evaluate the predictability of the obtained chemometric model of the proposed NIR method [27]

$$\mathrm{RMSECV}(\%) = \sqrt{\frac{\sum_{i=1}^{n}(C_{\mathrm{HPLC}} - C_{\mathrm{NIR}})}{\sum_{i=1}^{n} C_{\mathrm{HPLC}}}}$$

where C_{HPLC} is the amount of cefixime in as measured by the reference method , C_{NIR} is the amount of cefixime as measured by the proposed method and n is the number of samples. The chosen model had a RMSECV value of 1.27% and a coefficient of correlation ($r2$) of 99.83 as illustrated in Figure 3 which indicates good fitness and accuracy of the model.

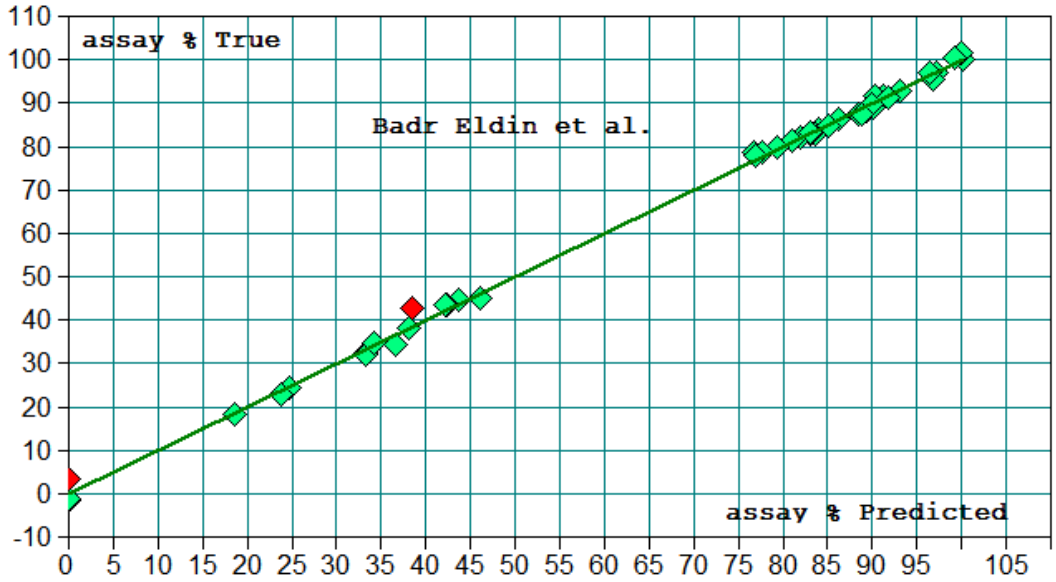

Figure 3. Regression of the calibration samples for NIR proposed method in cross validation

Agreement between the two methods for unknown samples

A simple plot of the results given by a method versus those of the other one is a useful mean to evaluate the validity of the proposed NIR method, however, the data points will usually be clustered near the line and it will be difficult to assess between method differences so that a plot of the difference between the methods against their mean is chosen. This plot of data may be more informative. Figure 4 shows the distribution of the differences against their mean.

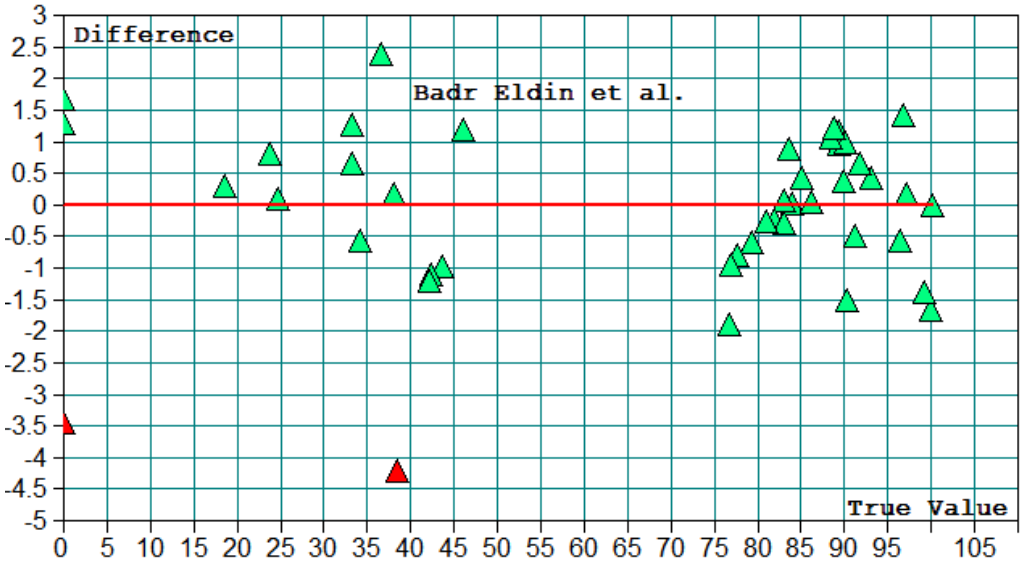

Figure 4. Illustration of distribution of the differences against their mean in cross validation

CONCLUSIONS

NIR spectroscopy has been shown to be a viable alternative to HPLC with UV detection for the assay of cefixime in capsules. If we have set up efficient calibration model, it could be used easily for rapid and accurate analysis of large number of samples. It is a non-destructive method, doesn't need sample pre-treatment or toxic solvents and reagents and thus it fulfill the green analytical chemistry aspects and suitable for on-line, in-line and off-line production control purposes. So this technique can replace the conventional HPLC techniques in some applications safely for developing more eco-friendly analytical methods realizing the green chemistry principles in direct and clear way.

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