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A Novel Method for the Elution of Sirolimus (Rapamycin) in Drug-Eluting Stents

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ABSTRACT

A sensitive and robust method for the determination of the elution of an active drug substance, sirolimus, from drug-eluting coronary stents was developed using a USP Apparatus 4 elution system. The closed-loop configuration of the elution apparatus and the small volume of eluent allow the low drug levels that elute from a single stent to be reproducibly monitored.

The in vitro elution profile obtained from USP Apparatus 4 over 24 h mirrors the 30-day in vivo porcine profile, providing an in vitro release method that captures the entire in vivo release profile of the stent in a shorter time. This method discriminates between common manufacturing and formulation product defects that were intentionally made. The method employs a novel elution medium containing an organic solvent, which allowed the in vitro elution curve to be fit to the in vivo porcine profile.

The method has been accepted by the FDA as a release method for the elution of sirolimus or rapamycin in cardiovascular stents.

INTRODUCTION

The drug-eluting stent (DES) is an important pharmaceutical combination product for the treatment of myocardial occlusion and prevention of restenosis. Restenosis, or arterial narrowing due to the formation of scar tissue, creates artery narrowing or total obstruction, which may require future interventional procedures. Drug-eluting stents minimize restenosis by releasing the drug during the critical time when restenosis is likely to form, in the weeks after a medical intervention. A typical DES formulation comprises an active drug mixed with one or more polymers and coated onto the surface of the bare metal stent. The polymers meter the drug release into the tissue over days or weeks. Depending on the formulation, the selection of polymer, and the drug substance used, elution and efficacy can be tailored. Since efficacy of the formulation is influenced by the release rate of the drug from the stent, the release rate must be carefully controlled to ensure product efficacy. An efficacious drug-eluting stent formulated with the appropriate drug and polymer can minimize restenosis and the need for resultant interventional procedures.

An important aspect of understanding and characterizing drug delivery systems is the release kinetics of the drug. Dissolution or elution testing, which is the terminology used for DES, is typically used as part of product characterization and as a finished product release test. In the past, significant work has been done to develop methods for evaluating the release of drug from other dosage forms (e.g., tablets, capsule, transdermal patches, implants) (*1–4*), but relatively little elution work has been done regarding drug-eluting stents (*5*).

One aspect of this work is to develop an in vitro elution test method that reflects the in vivo profile. An in vitro technique can generate elution profiles that can be compared to profiles generated from animal subjects. This comparison is a way of determining an in vitro–in vivo correlation (IVIVC). In the case of DES, initial testing is usually performed in porcine studies to determine the drug efficacy, safety, and performance. The in vivo results are used as the basis for the development of the in vitro method. Once the in vivo profile is generated, it is used as a basis for developing and optimizing the in vitro elution test method. A validated in vitro elution method with a profile that mimics the in vivo release profile can be used as a quality control tool in measuring product performance. An appropriate IVIVC may also reduce the requirement for additional preclinical and clinical studies.

This work demonstrates the development and validation of an elution test method which can be correlated to the product performance in an animal model. In addition, the method presented here is efficient in terms of analysis time and is easily run in a quality control laboratory as a routine method requiring an HPLC system with the typical detector and reagents.

In this paper, we demonstrate the steps undertaken to develop and validate an elution test method using compendial USP apparatus (*6*). We investigated parameters such as type of USP apparatus, choice of media and components, media pH, media temperature, and instrument speed.

The most important aspect of this investigation was to determine the most appropriate apparatus for the study of DES. Ultimately, we chose a method based on USP Apparatus 4, which uses an organic-based medium in this application. The parameters were refined to give a final method that provides results that are comparable to the in vivo profile. The validation process has demonstrated that the method is sensitive, accurate, and robust. Application of the method to manufacturing and formulation defect samples also proved the method is sensitive, discriminating, and

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*Figure 1. Sotax USP 4 CE7*smart *in a closed-loop configuration. Sample time points are manually taken at every time interval.*

robust. The final method and the validation are presented in this paper.

EXPERIMENTAL AND MATERIALS

Materials and Reagents

The elution medium was prepared using 2% ultra pure sodium dodecyl sulfate (SDS), in high purity water with 10% gradient-grade acetonitrile (ACN), and buffered to pH 4.5 with phosphate. The mobile phase was a mixture of 55:45:0.02 water/tetrahydrofuran (THF)/formic acid (v/v).

Instrumentation

A Sotax CE 7 Apparatus 4 instrument, Model 8000-2, equipped with 12-mm sample cell vessels was employed for the dissolution part. The system was configured for a closed-loop system to obtain greater sensitivity. Figure 1 shows the layout and configuration of the apparatus. An HPLC equipped with UV detection (Agilent) was used for the quantitation.

Dissolution Methodology and Condition

Previous experiments were performed using several types of elution media. A volume of 50 mL was employed to maximize the volume needed to prime each of the vessels and take advantage of the external media reservoir volume capacity. The method used a media flow rate of 25 mL/min, which provided the optimum pump performance with respect to priming and flow characteristics of the Sotax apparatus media pump. Samples were taken at several time intervals to capture the entire elution profile. The elution methodology and conditions are summarized in Table 1.

Analytical Methodology

Quantitation was performed with Agilent 1100 HPLC system equipped with UV detector. Parameters are listed in Table 1.

Table 1. Summary of Dissolution and HPLC Parameters

USP 4 Apparatus		**HPLC Parameters**	
Elution Media Composition	(2% SDS/10% ACN at pH 4.5	Column	Phenomenex Luna C18, 50 × 4.6 mm, 3 µm; Cat. No. 00B-4251-E0 or Phenomenex Gemini C18, 4.6 × 50 mm, 5 µm, Cat. No. 00B-4435-E0
Elution Media Volume	50 mL	Flow Rate/Isocratic	1.2 mL/min
Sample Cell Temperature	37 ± 0.5 °C	Injection Volume	50 µL
Media Flow Rate	25.0 ± 2.0 mL/min	Column Temperature	35 °C
Pull Volume	150 µL	Autosampler Temperature	Ambient
Sampling Time Points	15 min, 30 min, 45 min, 60 min, 2 h, 4 h, 6 h, 10 h, 24 h, 30 h	Detection Wavelength	UV at 278 nm
Sample Orientation	Samples were added to the sample cell without the need for a sample holder.	Mobile Phase	55:45, formic acid (0.02% v/v)/THF
		Run Time	7 min

RESULTS AND DISCUSSION

Elution Apparatus Selection

Initial work in developing an elution method specifically for DES made use of both noncompendial and compendial apparatus. Most conventional USP dissolution vessels, such as Apparatus 1 (basket) and 2 (paddle), require large volumes of media or significant modification, and are therefore not suitable for this DES application. In the noncompendial arena, many early attempts were made using simple extraction techniques. These techniques involved the use of various media in extraction vials with some form of agitation (shaker or vortexer) to elute the active drug, followed by quantitation by HPLC or spectroscopy. Others attempted to mimic the in vivo environment by using animal blood vessel, peristaltic pump, blood vessel tank, and so forth (*7*). Generally, the use of non-USP compendial apparatus is discouraged because of the difficulties in instrument calibration and in reproducibly duplicating the technology in other laboratories.

The most important aspect of this investigation was to determine the most appropriate apparatus for the study of DES. Evaluation of the various USP elution apparatus narrowed the choices to two candidates, USP 4 (flow through) and USP 7 (reciprocating). These two apparatus offer the advantage of using a low volume of medium, which is ideal for stent applications due to low drug level in the formulation (*8*). In addition, these apparatus can be

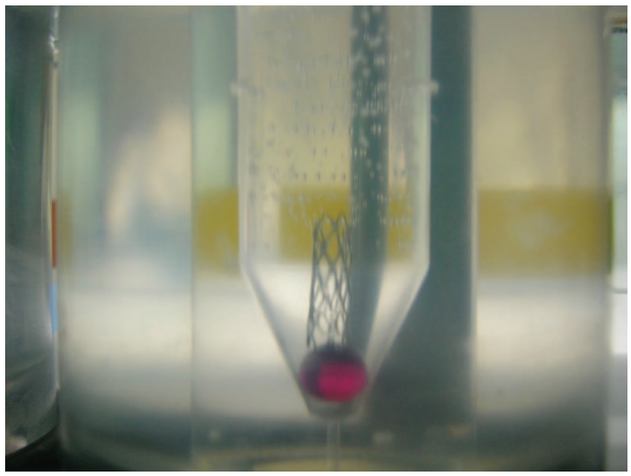

Figure 2. Close view of the Sotax flow cell with stent sample.

easily modified and tailored to accept stent samples of different sizes and geometries. These instruments were judged preferable for the DES samples studied and for promoting sensitivity in the quantitative analysis. Reproducible results when performing elution testing on products containing low concentrations demand good control of the sample path and meticulous control to prevent evaporation. These parameters are easily controlled by standardizing the tubing length and the media reservoir configuration as shown in Figure 2. Accurate media flow through the USP Apparatus 4 system ensures reproducible release of drug to the media vessels, and the closed-loop system minimizes evaporation. When USP Apparatus 7 equipment was used for this application, sample mixing proved to be an issue for the small volumes needed as well as lack of sensitivity with samples after a certain time. Media evaporation was also a factor that could be minimized but not eliminated with Apparatus 7. Analysis using USP Apparatus 7 generated elution profiles that were too slow to be suitable for releasing product in a single working shift in a QC environment. In a 24-h elution

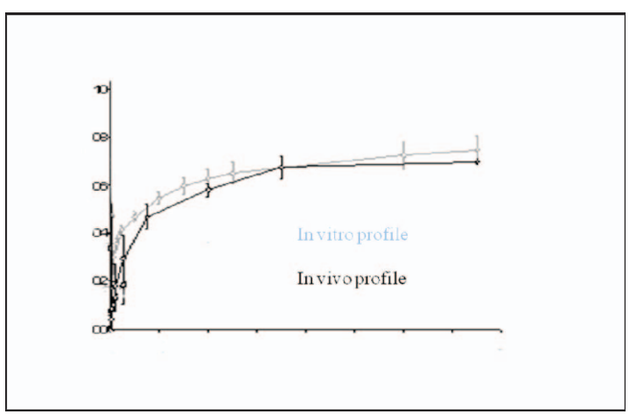

Figure 3. Comparison of the in vitro and in vivo profiles with time-scaling factor.

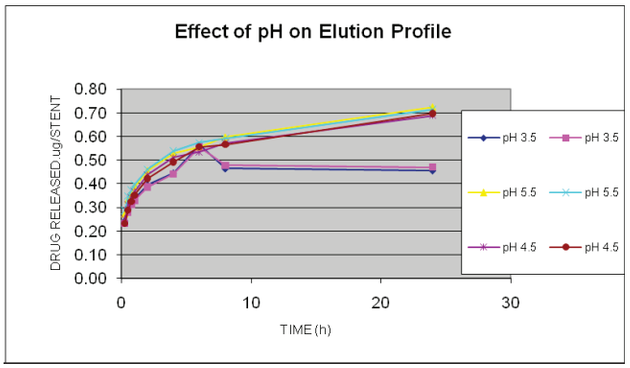

Figure 4. Effect of pH on elution profile.

run using Apparatus 7 with a Brij-35 concentration of 0.01% (w/w), only about 25% of the drug eluted, requiring additional days to attain the desired 80% elution profiles. With USP Apparatus 4, rapid elution analysis was possible. This was accomplished because of the flexibility of the system in setting media flow parameters. Varying the media flow allowed the elution rate to increase or decrease slightly, allowing for fine-tuning of the profile to match the in vivo profile. When dealing with DES, determining analysis time is crucial in minimizing medium evaporation and drug degradation. Lengthy in vitro elution analysis can promote drug or matrix degradation. For these reasons, USP Apparatus 4 was selected.

Elution Media Selection

Initial evaluation of elution media was carried out using a variety of buffered solutions. Use of aqueous-based elution media produced very slow elution profiles, which resulted in drug degradation during the analysis. Several media additives were evaluated, including ionic and nonionic surfactants such as SDS, Tween 20/80, and Brij-35. A mixture of an ionic surfactant, sodium dodecyl sulfate, and an organic solvent was chosen, which produced the closest match to the in vivo elution as shown in Figure 3. As part of method optimization, the concentration of SDS in the elution media was varied, but this produced no significant change in elution profile. Increasing the SDS

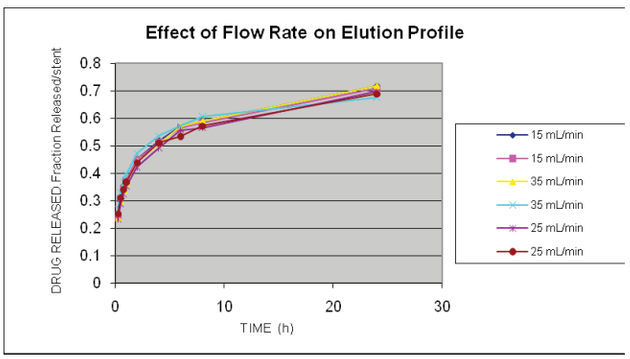

Figure 5. Effect of flow rate on elution profile.

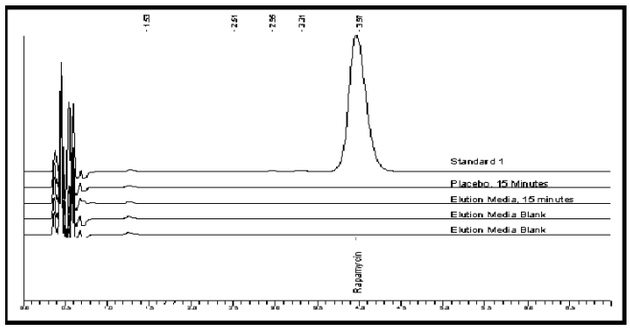

Figure 6. Specificity study at initial time point.

concentration above 5% caused precipitation of the surfactant and did not afford any advantage. As a result, the concentration of SDS in the elution medium was kept at 2%.

The pH of the elution medium was adjusted with 0.02 M sodium acetate buffer, pH 4.5. In general, buffering the elution medium provides better profile reproducibility. The elution buffer was chosen because it provided a robust buffer within the desired pH range (pH 4.5) without significantly changing the elution profile. Elution profile data from the pH study (Figure 4) shows a significant decline in the amount of drug released up to the 8-hour time point. This resulted in the degradation of rapamycin in the pH 3.5 medium over time. Therefore, the pH 3.5 elution medium is not a suitable medium for this method.

The addition of niacinamide and acetonitrile was investigated to reduce the amount of rapamycin degradation that occurred in the elution bath at 37 °C over the 24-h run time. Based on these initial results, it was determined that an appropriate stabilizing agent must be added to maintain the stability of the rapamycin throughout the elution analysis. Further experimental work showed that acetonitrile offered the best protection when added to the elution medium at about 10%.

Organic solvents were used to increase the elution rate and to match the in vivo profiles. The alcohol-based organic solvents that were initially tried promoted significant degradation of the active drug because of their basic nature. The addition of niacinamide and acetonitrile

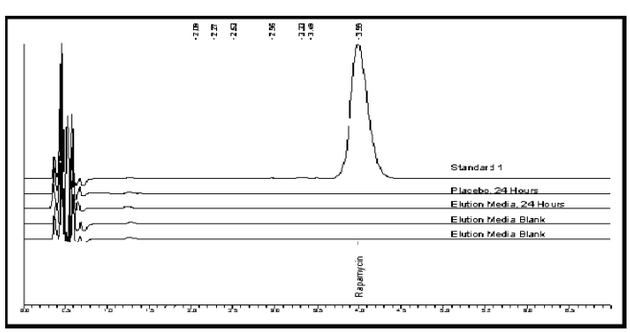

Figure 7. Specificity study at last time point.

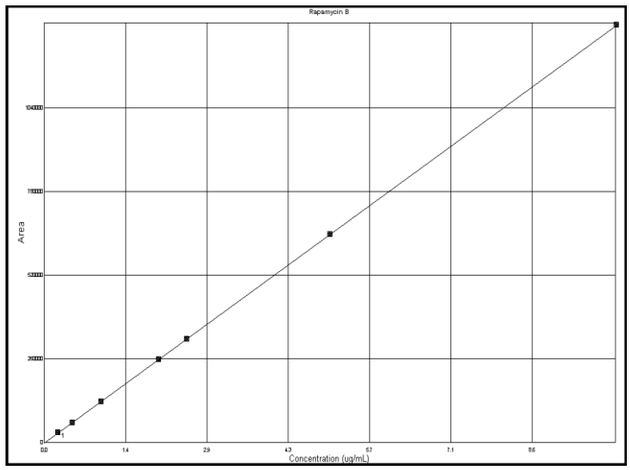

Figure 8. Linearity range of sirolimus from 0.25 to 10.0 µg/mL.

reduced rapamycin degradation, particularly acetonitrile. Use of an organic solvent extended the stability of the active drug in the elution medium throughout the elution run (*9*) for as long as 30 h with very little drug degradation. The addition of organic solvent also shortened the elution run time, which improved sample throughput.

Flow Rate Selection

With USP Apparatus 4, media flow rate must provide adequate flow to promote sufficient elution from the drug matrix and to provide adequate mixing of the drug in the medium. A slow flow rate causes problems with media priming during the initiation of a run and inadequate elution/mixing of the drug. A fast flow rate causes bubble formation and pump cavitation.

A flow rate setting of 25 mL/min was the best selection for this application. It takes advantage of the optimal

Table 2. Intermediate Elution Results from Three Independent Laboratories

Time Point	LAB 1 Intermediate Precision Summary (% Eluted)	LAB 2 Intermediate Precision Summary (% Eluted)	LAB 3 Intermediate Precision Summary (% Eluted)
15 min	28	29	28
30 min	34	36	35
45 min	38	42	39
1 h	41	44	42
2 h	48	52	49
4 h	55	60	56
6 h	60	65	61
8 h	64	68	65
10 h	66	71	67
24 h	72	77	74
30 h	73	78	76

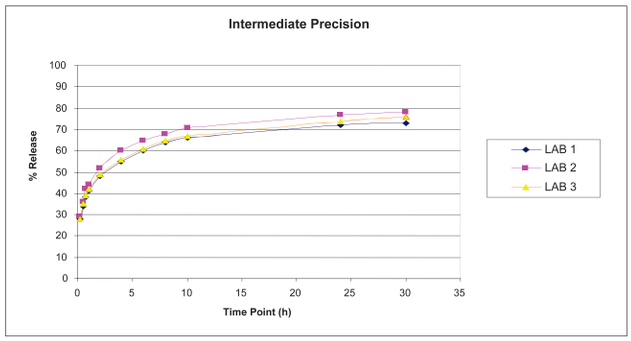

Figure 9. Intermediate precision plot showing the average elution results from three independent laboratories.

pump performance range and minimizes pump cavitation. Most importantly, initial media priming issues were less likely to occur. In the robustness experiments, it was determined that varying the flow rate at 25 ± 10 mL/min had no significant effect when compared with the normal results, as shown in Figure 5. It is important to note that this finding applies to this situation only, and other applications may require a narrower medium flow range.

Method Validation

Specificity

The evaluation of specificity is a crucial step in the development of an elution method, particularly one that runs for many hours. Throughout the elution run, it is important to minimize significant degradation, which can interfere with the quantitation of the main drug. Figures 6 and 7 show no observable interference with the sirolimus peak in the blank media and placebo stent at the initial and last time points.

Table 3. Method Robustness Variation and Criteria

HPLC Section	Elution Section
Varying Injection volume by ±10%	Media flow rate, 25 ± 10 mL/min
Varying THF concentration by ±11%	Sample cell temperature, 35 °C and 41 °C
Varying formic acid concentration by ±50%	Media concentration, 1% and 3%
Varying column temperature by ±14%	Stent orientation, horizontal vs. vertical
Varying flow rate by ±25%	
Varying UV detector wavelength by ±2%	
Varying autosampler temperature by ±5 °C	
Varying column lot (3 lots)	
Varying a column brand with similar phase (Luna vs. Gemini)	

Table 4. Description of Formulation and Manufacturing Defects

Group	Formulation Defects
A	Polymer X, polymer Y, and drug (normal)
B	No polymer X, polymer Y, and drug
C	Polymer X, no polymer Y, and drug
D	Polymer X, polymer Y, and no drug
E	High polymer X, polymer Y, and drug
F	Polymer X, high polymer Y, and drug
	Manufacturing Defects
1	Normal drug coating and polymer outer coating for entire length of stent (normal)
2	Normal drug coating and no polymer outer coating for entire length of stent
3	Normal drug coating and double polymer outer coating for entire length of stent
4	Normal drug coating, 2× polymer outer coating for ½ length of stent
5	Normal drug coating, 2× polymer outer coating for ½ length and normal for other half of stent
6	2× drug coating for ½ and 0 for other half of the length of stent, polymer outer coating for entire length of stent

Linearity

The coefficient of determination (r^2) that was generated for the linear curve in Figure 8 was greater than 0.9999. The linear range should be established to bracket the elution concentrations expected from the smallest to the largest stent size.

Accuracy

Accuracy was determined at the 0.25 and 10.0 µg/mL levels at the first time point (15 min) using placebo stents containing the appropriate coated polymers minus the drug, which was spiked during the elution run. A recovery

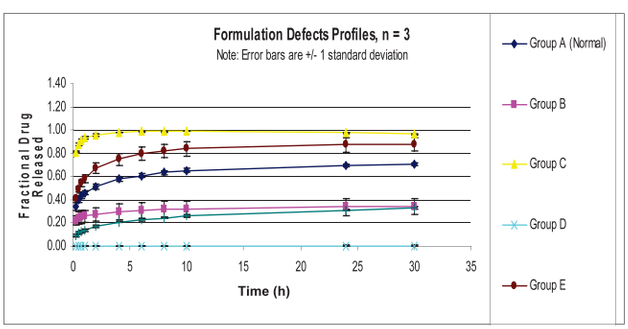

Figure 10. Discrimination of formulation defect samples.

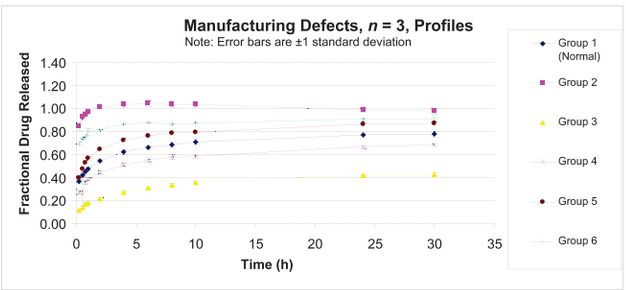

Figure 11. Discrimination of manufacturing defect samples.

of 99% was achieved for both levels. At the 24-hr time point, the recoveries for the 0.25 and 10.0 µg/mL levels were 96% and 95%, respectively.

Method Precision

Method precision results from various laboratories were typically less than 1.0% RSD. Laboratory intermediate precision was performed by three independent groups as shown in Table 2 and Figure 9. Results show that one must consider the nature of the individual stent. The percent dissolved mean result at each time point from three independent analysts is less than or equal to 10%.

Solution Stability

The standard solution was stable for seven days and the working standard for three days at ambient condition. Sample solution stability was determined for two days. Determining solution stability is crucial in the analysis, particularly when a laboratory investigation is warranted.

Method Robustness

Method robustness was determined for both the elution and the quantitative sections of the method. The HPLC and elution robustness parameters were varied as described in Table 3.

Method Discrimination

One of the requirements for an elution method is the ability to be sensitive and discriminatory towards manufacturing and formulation defects. An elution method that can detect any of these defects is what is required in a quality control laboratory. This method is able to distinguish profile differences due to the various defects, as seen in Table 4 and Figures 10 and 11. The polymer and drug ratios were intentionally modified to simulate possible formulation and manufacturing defect scenarios.

IVIVC

In vivo–in vitro (IVIVC) correlation was achieved with this method. The 24-hr in vitro profile was compared with the 30-day in vivo porcine profile, which shows correlation as shown in Figure 3.

CONCLUSION

It was important that the method presented above, which was the first of its kind, be tested thoroughly and comprehensively. The method is sensitive, accurate, reproducible, and robust for determining the elution profile of drug-eluting stents (*10*). The method discriminates manufacturing and formulation defects.

The USP 4 Apparatus profile over 24 h mirrors the 30-day in vivo porcine profile, providing an in vitro release method that captures the entire release profile of the stent. It allows the measurement of an elution profile within a fraction of the real-time in vivo study; thus, it is suited for a quality control laboratory environment.

The method has been accepted by FDA as a release method for the elution of sirolimus in cardiovascular stents.

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