Therapeutic Drug Monitoring by LC/MSD - Clozapine, an Example

Application

Pharmacology

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Abstract

Therapeutic drug monitoring is performed by LC/MS on a routine basis at St. Olav Hospital. Over 200 drugs are analyzed for both identification and quantification using this technique. This is made practical by high-throughput analysis and the use of carefully applied methodology. The method for clozapine and its metabolite, desmethylclozapine is given as an example. The advantages over immunoassay are briefly discussed.

Introduction

Therapeutic drug monitoring (TDM) has been available for many years for drugs with a narrow therapeutic index (for example, digoxin, theophylline, and lithium). Analyses for most of these drugs have been performed with immunological techniques. For many years clinicians believed that there was no need to perform TDM on a routine basis for the majority of other drugs. However, during the past few years there has been increased focus on, and a growing awareness of, the interindividual variability in response to drug exposure. This variability may be due to pharmacodynamic, pharmacokinetic, environmental (food, tobacco smoke, ethanol), psychological (insight into the disease), and compliance factors. The lack of ways to correct for many aspects of this variability, other than pure clinical judgement, has evoked a renaissance for TDM. Initially the focus of the "new" TDM was directed towards treatment of psychiatric and neurological disorders, but TDM is now expanding into other medical areas.

TDM at St. Olav Hospital

The Department of Clinical Pharmacology at St. Olav Hospital, Trondheim, routinely monitors more than 200 different drugs, almost exclusively by liquid chromatography/mass spectrometry (LC/MS). For example, there are methods available for more than 20 drugs used for treatment of schizophrenia and other psychoses. One of the drugs in this group that is routinely monitored is clozapine, which is analyzed in about 200 samples per month. Other drugs in the same group include, but are not limited to, olanzapine, quetiapine, ziprasidone, risperidone, sertindole, amisulpiride, haloperidol, perphenazine, zuklopentixol, fluphenazine, chlorpromazine, thioridazine, and chloroprotixene. Similar repertoires are also available for other groups such as antidepressants and mood stabilizers.

Clozapine

Clozapine is mainly used against therapy resistant schizophrenia. Clozapine's mechanism of action is not completely understood, but it is assumed that a combination of a weak dopamine antagonism together with combined serotonin agonism and antagonism are the most important mechanisms. Clozapine is metabolized to numerous metabolites, mainly by the cytochrome P450 (CYP1A2) enzymes, and the half-life in blood usually is within the range of 6–36 hours. Some data indicate that individuals with a high level of desmethyl-clozapine are more susceptible to develop agranulocytosis; that is a potentially lethal adverse event.

Experimental

In the TDM-service, clozapine and one of its metabolites, desmethylclozapine, are measured in serum by the Agilent Technologies 1100 LC/MSD. The LC/MS with a single quadrupole provides both the selectivity and sensitivity needed for this analysis. Both the ruggedness of the methodology and the instrumentation make it a cost effective process for continuous operation. The use of in-house instrument engineers and meticulous documentation of maintenance operations along with the records obtained from operation 24 hours a day, 7 days a week provide "up-time" of greater than 95%.

All analytical results are transferred to a database. The actual concentration in the serum is interpreted by a MD (specialist in clinical pharmacology). The interpretations usually consist of an evaluation of the concentration/dose relationship in the actual sample together with evaluation of age, possible drug-drug interactions, possible adverse events if focused by the clinician, etc. versus therapy success or lack of success. New measurements are always compared with previous results in the database. The overall impression is that TDM is an extremely valuable tool for the clinician in the decision making process.

Table 1 gives the extraction procedure for the preparation of blood serum for the analysis of clozapine and its metabolite.

Table 1. Procedure to Extract Clozapine and its Metabolite from Blood Serum

Step	Action
1.	Take 0.5-mL serum (standard, QC or sample).
2.	Add 50 µL of 10-nM flurazepam [internal standard (ISTD) for clozapine].
3.	Add 50 µL of 10-nM periciazin (ISTD for desmethylclozapine).
4.	Mix (short).
5.	Add 4 mL of hexane: n-butanol: acetonitrile (93:5:2).
6.	Mix 5-min on rotary mixer.
7.	Centifuge 3000 rpm 5 min.
8.	Transfer organic phase.
9.	Evaporate under stream of air at 40 °C.
10.	Reconstitute in 50-µL methanol.
11.	Transfer to vial with 150 µL insert and cap with crimp-on cap.

Two internal standards (ISTDs) are used, flurazepam and periciazin. The structures of each are shown in Figure 1.

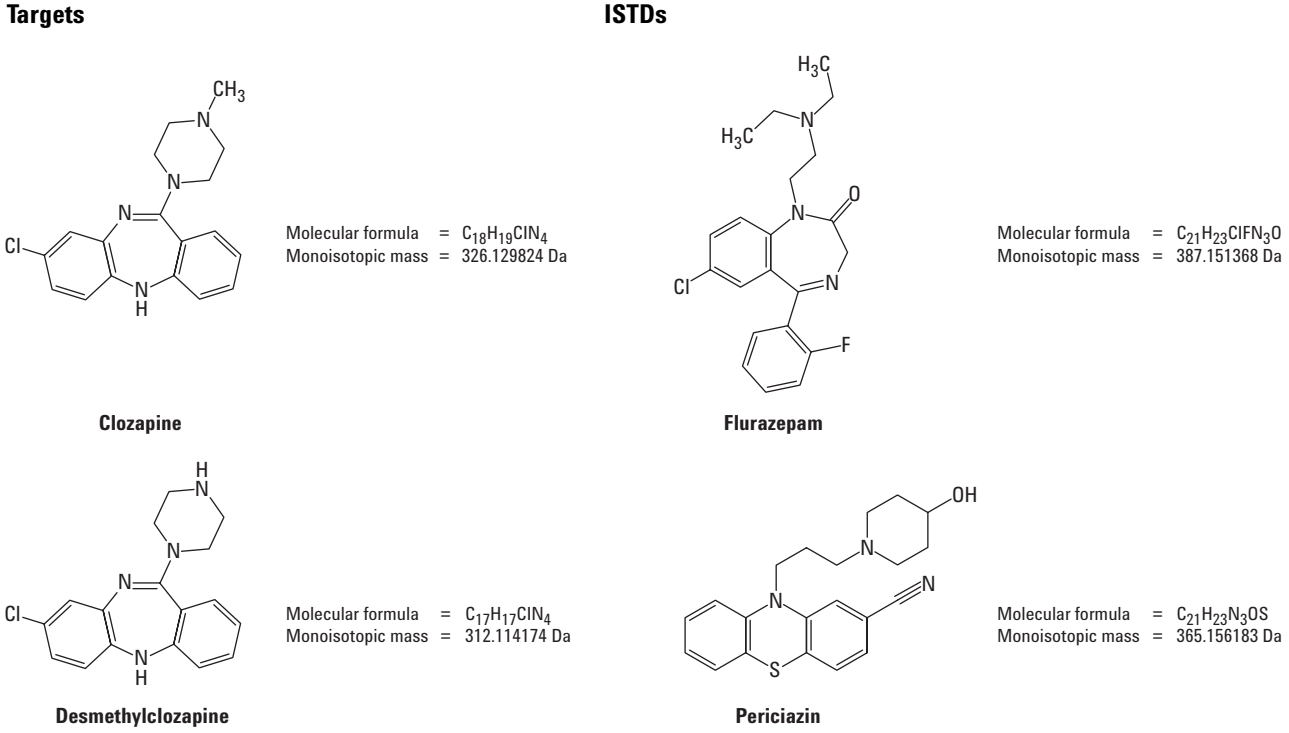

Figure 1. Structures of clozapine, desmethylclozapine and their ISTDs (shown to their right).

Instrument conditions for the analysis are shown in Table 2.

Table 2. Instrumental Conditions for the Analysis

LC/MS Analysis			
LC Conditions			
Instrument	Agilent 1100 LC/MSD with Quaternary pump and electrospray		
Column	ZORBAX SB-C18, 4.6 mm × 30 mm × 3.5 µm, p/n 833975-902		
Mobile phase	Methanol: 50-mM ammonium acetate in water (60:40)		
Flow rate	1 mL/min		
Standard automatic liquid sampler (ALS) with needle wash			
MS Conditions			
Injection Volume	2.5 µL		
Nebulizer	25 psi at 350 °C		
Drying gas	9 L/min		
	RT (min)	**Target**	**Qualifier**
Clozapine	3.0	327.0/frag 100 V	270.0/frag 150 V
Desmethylclozapine	1.3	313.0/frag 100 V	270.0/ frag 150 V
Flurazepam	2.2	388.1/frag 100 V	
Periciazin	1.9	365.5/frag 100 V	

Calibration is done with zero serum using spiked concentrations of calibration standards (clozapine and desmethylclozapine): 0, 25, 100, 500, 1000, 2000, 3000, and 4000 nM. The calibration curves use a quadratic fit (because of the wide range of concentration) with r^2 >0.999. Quality control samples are prepared with zero serum using spiked concentrations of QCs (clozapine and desmethylclozapine): 500, 500, 1000, 1000, 3000 and 3000 nM. The QCs are run randomly distributed among the samples.

Results

Figure 2 shows the selected ion chromatograms of the target compounds with qualifying ions and their ISTDs for the 25 nM standard.

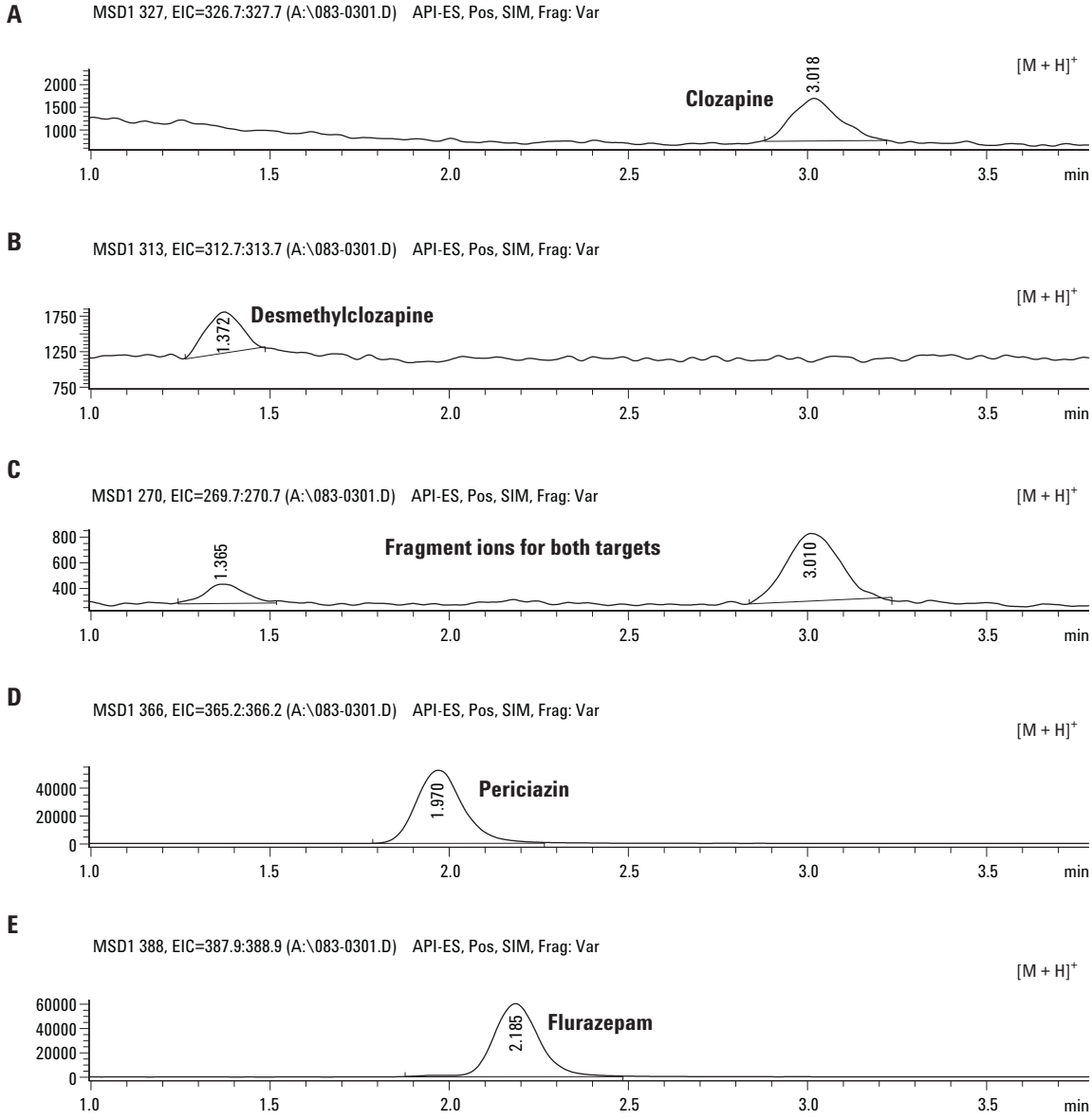

Figure 2. Selected ion chromatograms for 25 nM clozapine, desmethylclozapine, and their ISTDs. All chromatograms show the $[M+H]^+$ ions. Figure 2A is derived from clozapine, 2B from desmethylclozapine, 2C from fragments from both target compounds (after neutral loss of 1-N-methylaziridine), 2D from peraciazin, and 2E from flurazapam.

Up-front collision-induced-dissociation (CID) is sufficient to produce a qualifier ion to increase the specificity of the analysis. This is the lowest standard in serum and represents a concentration typically below normal therapeutic monitoring levels. There is variation of the serum matrix from one sample to another but that variation is not sufficient to have any impact on the analysis.

Figure 3 shows a blank containing the ISTDs and Figure 4 presents the results of a patient sample at a typical lower level (for true patient samples). In this patient sample, the clozapine was measured at 366 nM, and the metabolite at 365 nM. Note how clean the signals are with no interference. The methods demonstrated were robust for a period of 5 years.

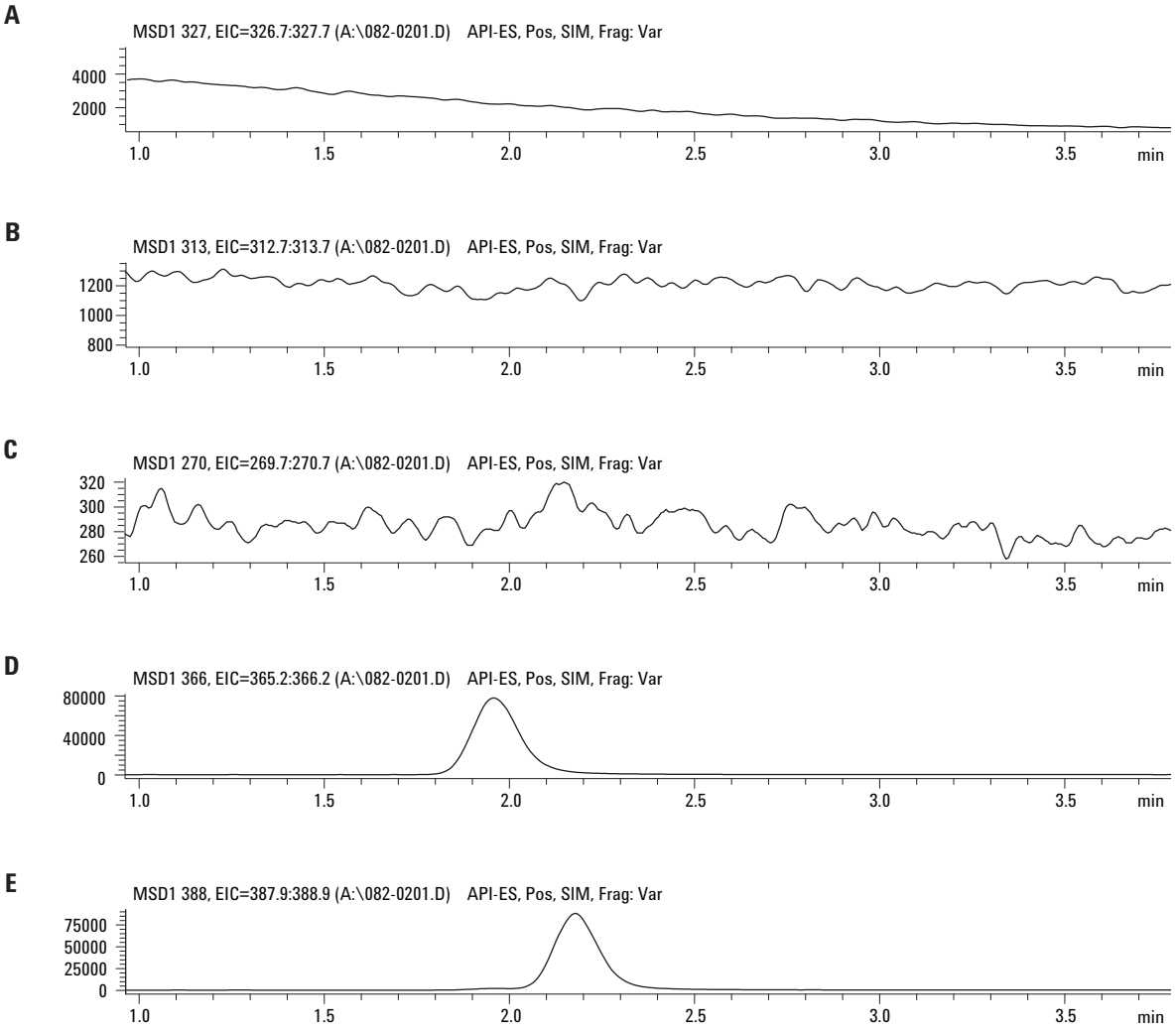

Figure 3. Extracted ion chromatograms (EIC) of blank clozapine in blood serum with ISTDs.

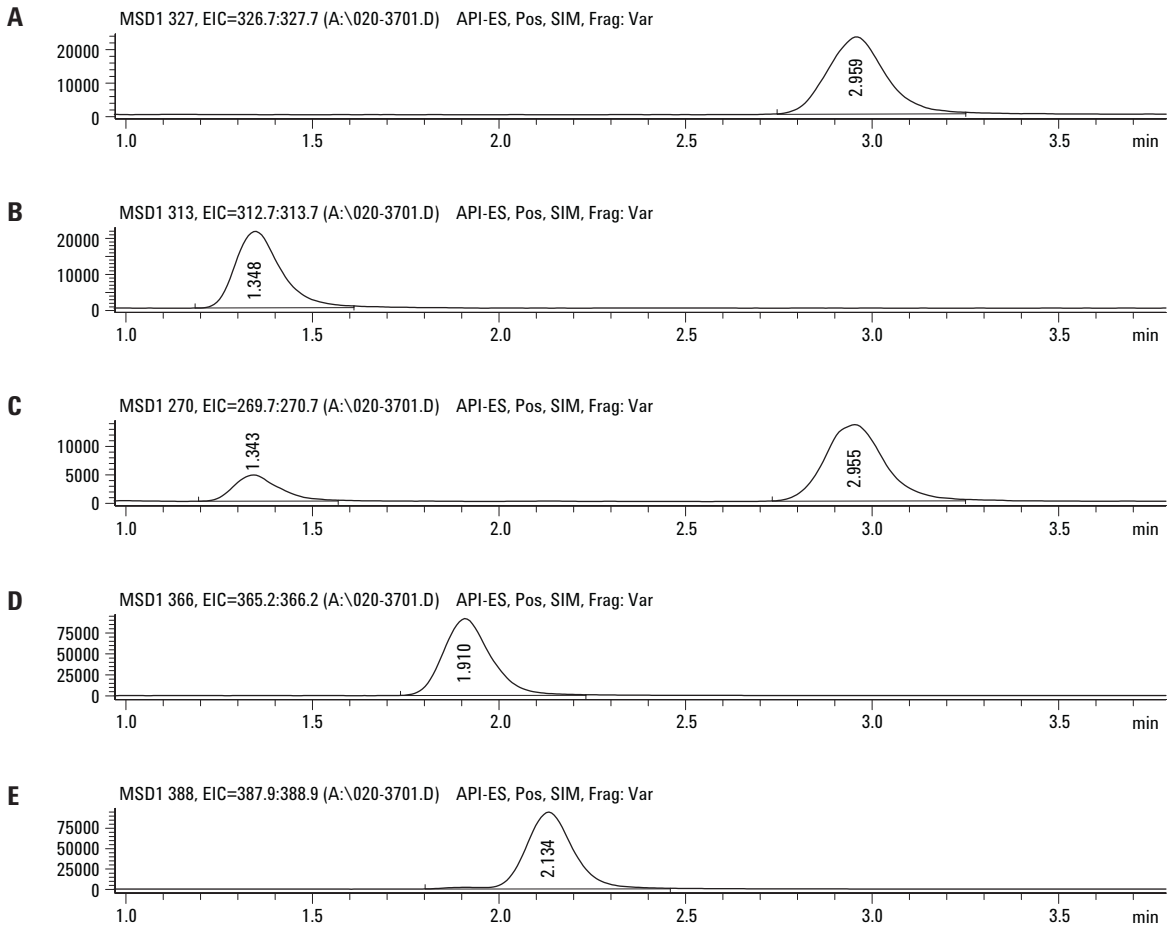

Figure 4. EIC of patient sample at a typical low level.

Reproducibility

True day-to-day reproducibility with different instruments and different operators is obtained through the evaluation of the QC samples. These are shown in Table 3, as a 3-month rolling average.

Table 3. True Reproducibility of Analysis (3-month rolling average)

	25 nM	500 nM	3000 nM
Clozapine	6.1%	6.1%	7.8%
Desmethylclozapine	9.1%	8.0%	7.6%

Table 4 shows a 1 day example of the QC results at the different levels.

Table 4. True Reproducibility of Analysis (1 day example)

	500	1000	3000
Clozapine	596	1068	3361
	543	1068	2935
Desmethylclozapine	462	1026	3345
	534	992	2904

Once per month QC samples from Heatcontrol/ Cardiff Bioanalytical Services Ltd. are analyzed and a report generated. A 12-month control chart of the Bias Index Score (BIS) is shown in Figure 5. The BIS is defined as the difference of the laboratory's measurement from the consensus mean scaled in terms of a chosen common coefficient of variation for all participants. If the exact coefficient of variation is used, a BIS value of 300 corresponds to 3 standard deviations (SDs). External quality control samples are used for any TDM analyte that is available.

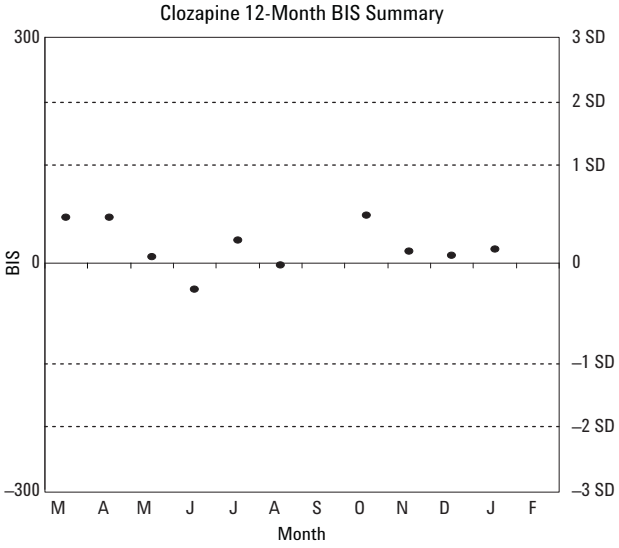

Figure 5. Control chart for external quality sample.

Conclusion

Through the use of well developed and validated methodology, carefully documented procedures followed by highly skilled and trained operators (technicians or engineers), and rigorous QC incorporated into good laboratory practices, this lab routinely performs therapeutic drug monitoring, clozapine as an example, with LC/MS running 24 hours a day, 7 days a week. For clozapine, typical serum levels for patients range from 450 nM to 3000 nM.

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