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Research Article

Rapid LC-MS/MS method for determination of scopolamine in human plasma

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Abstract

Sensitive, simple, and fast LC-MS/MS method for the determination of Scopolamine in human plasma was developed and validated. Liquid-Liquid extraction technique was used for sample preparation. Cyano bonded phase column (150 × 4.6 mm, 5 µm) was used for the separation with an isocratic elution of ammonium format buffer:methanol (60:40) mobile phase at a flow rate of 1 $ml.min^{-1}$ over 3.8 min run time. Scopolamine and [$^{13}C_2H_3$]-Scopolamine, as internal standard, were detected and quantified in positive ion mode via MRM at m/z 304/138 and m/z 308/142, respectively. The developed method was validated according to FDA and EMA guidelines. The standard calibration curve was linear over the concentration range of 3.03–315.76 $pg.ml^{-1}$ ($r^2 = 0.999$). The intra-day and inter-day precision was in the range 1.28–10.46% and accuracy 96.89–110.53%. The recovery of analyte and IS was 78.63% and 76.21%, respectively. Scopolamine in plasma was stable at benchtop (short term) for 18 h, in autosampler tray for 43 h, in instrumentation room for 43 h (post-preparative), after 4 freeze-thaw cycles (−70 °C), and 3 days in the freezer (−70 °C). The validated method was successfully applied to a bioequivalence study of scopolamine transdermal patch of 1 mg for 3 days for 16 healthy Jordanian volunteers.

Keywords

LC–MS/MS, scopolamine, bioanalytical, pharmacokinetics, bioequivalence

Introduction

Scopolamine, also known as hyoscine, is a natural alkaloid drug obtained from several plants such as *Datura stramonium*, *Hyoscyamus niger*, *Scopolia carniolica* and many other plants from the family Solanaceae, plants from this family produce natural toxic belladonna alkaloids (i.e. Scopolamine) as a protective mechanism (Pergolizzi et al. 2012). Scopolamine is an alkaloid drug with a structure shown in Fig. 1, which works as a nonselective muscarinic antagonist by inhibiting acetylcholine binding to a muscarinic receptors, this generate peripheral antimuscarinic characteristics, central sedative, antiemetic, and amnestic actions (Zhang et al. 2017). The parasympatholytic scopolamine, which is structurally related to atropine, is used in situations that need reduced parasympathetic activity, particularly for its effects on the eye, gastrointestinal system, heart, salivary, and bronchial secretory glands, and in rare cases for a CNS impact (Renner et al. 2005). As a result, scopolamine is best used for premedication and antiemetic effects before anesthesia (White et al. 2007). Scopolamine recommended as the most effective single

drug used to prevent motion sickness and vomiting (Cronin et al. 1982). Sleepiness, impaired vision, dilated pupils, and dry mouth are all common adverse effects of Scopolamine (Honkavaara et al. 1995; Pergolizzi et al. 2012). However, the agent's effectiveness when taken orally or parenterally has been limited due to it is short duration of action and it is high incidence of adverse effects, thus a Scopolamine transdermal formulation has been developed (Atkins 2003; Whelan and Apfel 2013; Bailey et al. n.d.).

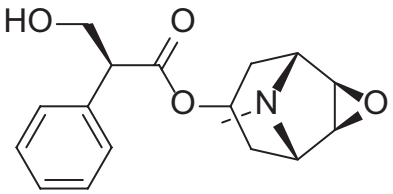

Figure 1. Chemical structure of Scopolamine.

Many analytical methods had been developed and validated to quantify Scopolamine in human plasma as a single compound or simultaneously with other alkaloids. Scopolamine in urine and plasma was measured using a semi-automated solid-phase extraction followed by a radioreceptor test (Bosman et al. 1997). For urine and plasma, the method detection limits are 550 pg.ml^{-1} and 16 pg.ml^{-1}, and quantification limits of 610 pg.ml^{-1} and 38 pg.ml^{-1}, respectively. Another LC-MS/MS method for determining scopolamine butyl bromide in human plasma was developed and validated (Manfio et al. 2009). The internal standard for this method is propranolol, which is extracted in a single step from liquid to liquid. Scopolamine in human plasma has also been determined using a solid-phase extraction technique and LC-MS/MS (Swaminathan et al. 2019). In a human pharmacokinetic investigation, the test was used to assess the levels of scopolamine in human serum after it was given as a transdermal or intravenous bolus. Koželj and his team have developed LC–MS/MS method using protein precipitation with acetonitrile to determine scopolamine simultaneously with atropine in 100 µL human plasma, where a very low lower limit of quantification (LLOQ) of 0.10 ng/mL was achieved for both compounds (Koželj et al. 2014). Additionally, another LC–MS/MS method was developed and validated for quantification of atropine, anisodamine, and scopolamine in rat plasma, to investigate the pharmacokinetics of tropane alkaloids in *Hyoscyamus niger* L. (Zhang et al. 2014). a capillary zone electrophoresis separation coupled to TOF-MS detector was developed for the analysis of strychnine, brucine, anisodamine hydrobromide, atropine sulfate, anisodine hydrobromide, and scopolamine hydrobromide in human plasma and urine, (Yu et al. 2012).

In this work, an LC-MS/MS method has been developed and fully validated for the determination of Scopolamine in human plasma using K_3EDTA anticoagulant. The method was sensitive and robust, yet it was simple to be applied in any ordinary bioanalytical laboratory. The method was successfully applied to a bioequivalence study of scopolamine transdermal patch formulations involving 16 healthy Jordanian volunteers.

Experimental

Chemicals and reagents

Scopolamine hydrobromide and internal standard (IS) ([$^{13}C_{,2}H_3$]-Scopolamine oxalate salt) were obtained from ALSACHIM (Illkirch-Graffenstaden, France). LC-MS grade Methanol, n-Hexane, and Ethyl acetate were obtained from Honeywell (Charlotte, NC, United States). Sodium hydroxide and Formic acid were obtained from ISOLAB (Eschau, Germany). Ammonium format was obtained from Fischer (Schaffhausen, Switzerland). HPLC Water was obtained from Avantor (Radnor, PA, United States). Human K_3EDTA plasma obtained from Pharmaceutical Research Unite (PRU) clinical site (Amman, Jordan).

Instrumentation and chromatographic conditions

Chromatographic separation was achieved using a Shimadzu Nexera XR system (Kyoto, Japan) using cyano column (150 × 4.6 mm, 5 µm) obtained from ACE (Reading, UK). The auto-sampler temperature was 6 °C. The analyte and IS were separated with an isocratic elution of ammonium format buffer:methanol (60:40) mobile phase, using flow rate of 1 ml.min^{-1}. The mass spectrometric data were collected on a Shimadzu LC-MS 8060 (Shimadzu, Japan) with a triple quadrupole mass analyzer. Multiple reactions monitoring (MRM) mode and positive mode of ESI interface were intended for Scopolamine (m/z 304/138) and [$^{13}C_{,2}H_3$]-Scopolamine (m/z 308/142). The separation of analyte spray droplets was accomplished by adjusting the nitrogen gas at a flow rate of 3 L.min^{-1}. The analysis data were obtained by Lab solution software, version 5.9.1 from Shimadzu (Kyoto, Japan).

Preparation of standards and quality control samples

Standard solutions of scopolamine were prepared from stock solution (160 µg.ml^{-1}) and IS (80 µg.ml^{-1}) in methanol which stored at –20 °C. All standard solutions of Scopolamine and IS were prepared by diluting the stock solution using methanol. Quality control (QC) samples and calibration standard solutions were prepared by spiking blank plasma with Scopolamine at concentrations of 3.03, 6.06, 16.17, 40.42, 78.94, 189.46, 284.18, and 315.76 pg.ml^{-1}. QC samples concentrations were 3.03 pg.ml^{-1} for lower limit of quantification (LLOQ), 9.09 pg.ml^{-1} for low quality control-1 (QCL-1), 60.63 pg.ml^{-1} for low quality control-2 (QCL-2), 126.30 pg.ml^{-1} for middle quality control, (QCM), and 236.82 pg.ml^{-1} for high quality control (QCH).

Sample preparations

For extracting Scopolamine and IS from human plasma, Liquid-Liquid extraction was performed using ethyl acetate and n-hexane (70:30) as extraction solvent. 500 µL spiked human plasma sample and 50 µL [^{13}C,

$^{2}H_{3}$]-Scopolamine was transferred to an Eppendorf micro tube (2 ml) then vortex-mixed for 30 s then 100 µl of NaOH solution (0.2 M) was added and vortex for 30 s. After that, 3.0 ml of extraction solvent was added to the sample and vortexed for 5 min and centrifuged (5 min @ 3000 rpm, 2–8 °C). The organic solvent was decanted and evaporated under vacuum for 15 min, then it was reconstituted with 300 µL mobile phase. Finally, 180 µL of aliquots were injected into the LC-MS/MS unit.

Method validation

The developed method was validated according to the in the FDA and EMA guidelines. Selectivity, sensitivity, linearity, matrix effect, precision, accuracy, integrity, stability, and dilution recovery were all evaluated. The selectivity was evaluated by injection of eight different lots of hemolyzed and hyperlipidemic blank plasma. Caffeine, Paracetamol, Diclofenac, ascorbic acid, Nicotine, Aspirin, and Ibuprofen were all examined as potential concomitant medication interference. The standard calibration curves were evaluated by plotting eight different levels. Sensitivity was tested by analyzing six triplicates of LLOQ against a calibration curve. The matrix effect was evaluated at two different levels (QCL-1 and QCH) using eight different lots of blank plasma including hemolyzed, and hyperlipidemic. For intra- and inter-day precision and accuracy six determinations at LLOQ, QCL-1, QCL-2, QCM, and QCH were extracted and assessed against the calibration curve. The peak area of non-extracted standard was compared to extracted standard to establish Scopolamine and IS recoveries. The bioanalytical method's recovery was calculated for Scopolamine at three concentration levels (QCL-1, QCH, and QCM), and for IS at the QCH concentration. Dilution integrity at a concentration of two times the concentration of QCH. Six replicates of each concentration were tested. Stability tests were evaluated to the stock solutions and plasma samples to assess Scopolamine stability under various conditions. The stability of stock solution was evaluated in two conditions: at room temperature and –20 °C, by comparing the area of Scopolamine in stability sample to the area in freshly prepared solution. Six duplicates at QCL-1 and QCH levels were used to examine bench top stability (18 h), freeze-thaw (four cycles), autosampler stability (43 h), and long-term stability (3 days).

Pharmacokinetic study

The pharmacokinetic parameters of Scopolamine were measured in sixteen healthy Jordanian volunteers in a by measuring the rate and extent of scopolamine after using scopolamine transdermal patch. This study was approved by the Institutional Review Board/Independent Ethics Committee (IRB/IEC) (Bošnjak 2001). Informed consent containing the purpose, procedures, risks that could be happening and all information needed about this study was taken from all subjects as directed according to the Declaration of Helsinki for biomedical research. Each volunteer received one transdermal patch (2.5 cm^2) containing approximately 1 mg scopolamine and it was applied for 72 hours. 8 mL of blood samples were collected from a forearm vein in labelled K_3EDTA blood tubes at (predose) and at 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 6, 7, 8, 9, 10. 12, 14, 16, 20, 24, 30, 36, 48, 60, 72, 84, 96, 108, and 120 hours post dosing. Samples were centrifuged (3500 rpm for 10 min) and supernatant was transferred to pre-labeled polypropylene tubes then stored in a freezer at –70 °C. WinNonlin version 8.3 software form Scientific Consulting Inc. (Asheville, NC, USA) was used to assess all pharmacokinetic parameters.

Results and discussion

Method development

Both positive and negative ionization modes were investigated for Scopolamine. The positive mode response was more suitable than negative mode. Chromatographic parameters were optimized to achieve high resolution and improved scopolamine signal intensity yet maintaining a short run time. Scopolamine detection was enhanced after addition of formic acid to the mobile phase. Different ratios of mobile phase were evaluated, and the optimum ratio was 60:40 of ammonium format buffer and methanol. Many columns stationary phases and brands were tested and ACE Cyano (150 × 4.6 mm, 5 µm) was the optimum one and was used for chromatographic separation. The retention time of Scopolamine and IS was about 2.3 min. Liquid-Liquid extraction approach was used for sample preparation as it facilitates high selectivity separation and cleaner matrix than direct protein precipitation.

Method validation

Selectivity

Selectivity reflects the degree of interference of Scopolamine and IS with endogenous plasma components. It was demonstrated by examining chromatograms from processed blank plasma samples and endogenous components in drug-free plasma. no interference with analyte peak was observed as illustrated in Fig. 2. Likewise, commonly used medications (caffeine, paracetamol, diclofenac, ascorbic acid, nicotine, aspirin, and ibuprofen) also showed no interference (data not showed).

Sensitivity

The sensitivity of the developed method was determined using LLOQ sample by evaluating signal to noise ratio in order to ensure more than five times response as compared to blank. The value of 3.03 $pg.ml^{-1}$ was chosen as the LLOQ for Scopolamine. At LLOQ concentrations, Scopolamine precision and accuracy were determined to be 10.1% and 102.3%, respectively, which revealed a good sensitivity of the method.

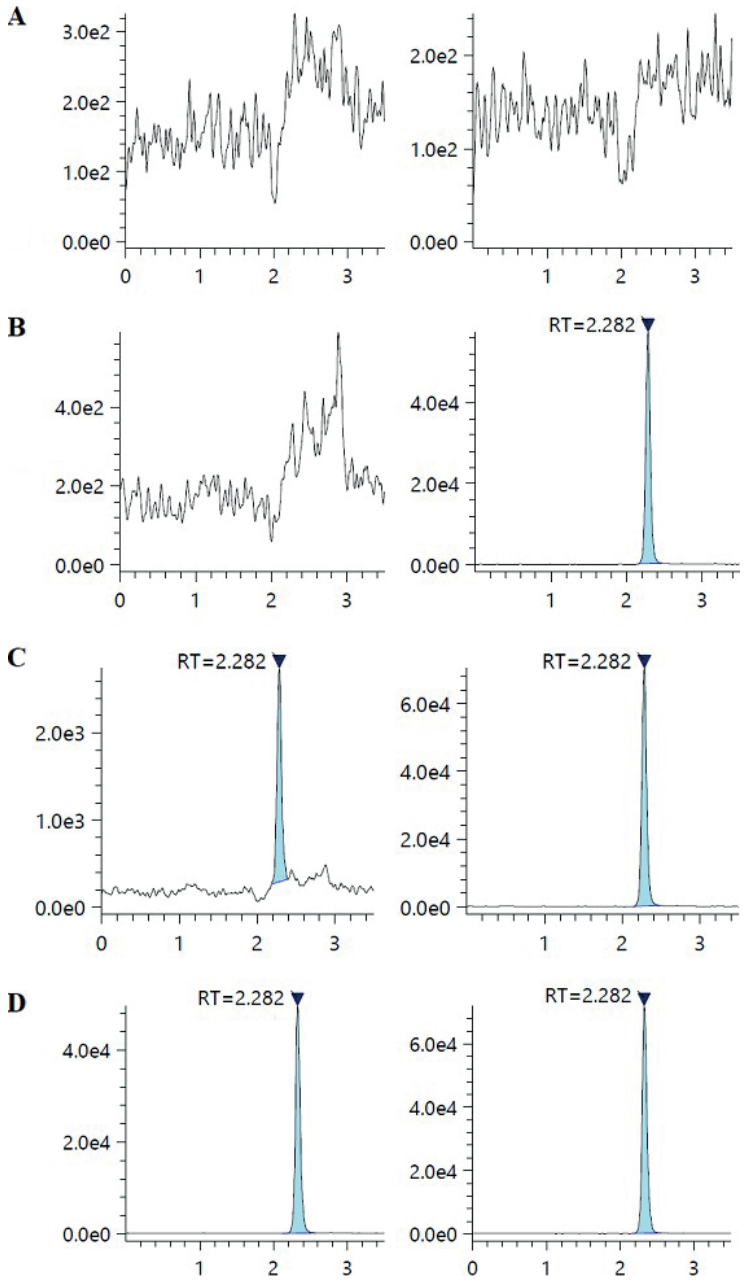

Figure 2. MRM ion-chromatograms of Scopolamine (m/z 304/138) and [13C,2H3]-Scopolamine IS (m/z 308/142) in (**A**) blank plasma (without analyte and IS), (**B**) blank plasma with IS, (**C**) Scopolamine at LLOQ with IS and (**D**) real subject sample at Cmax after administration of 1 mg transdermal patch of Scopolamine.

Matrix effect

Matrix effect quantitative evaluation of Scopolamine and IS, the peak area response for aqueous samples (representing 100% recovery at QCL-1 and QCH levels), were compared to the extracted post-spiked blank with aqueous samples QCL-1 and QCH, respectively. The precision for Scopolamine was found to be 1.94%, and 0.10% at QCL-1 and QCH concentration, respectively, indicating no significant matrix effect was detected in the method.

Linearity

Over the concentration range of 3.03–315.76 pg.ml^{-1} the method was found liner. A regression equation with a weighting factor (1/x^2) of Scopolamine to the IS concentration provided the best match for the concentration–detector response relationship for scopolamine in human plasma. The calibration curves created during validation have a mean correlation coefficient of 0.999.

Precision and accuracy

The accuracy of the developed method was evaluated in terms of % Recovery, and it was found to be ranged from 96.89 to 107.57% % for intra-day accuracy and from 97.74 to 110.53% % for inter-day accuracy. The inter- and intra-day precision were evaluated using 18 replicates and six replicates, respectively. The CV% for both precisions was in the range of 1.28 to 10.46%. The results summarized in Table 1.

Table 1. Intra-day and inter-day precision and accuracy for scopolamine.

QC Level	Intra-day (n = 18)				Inter-day (n = 6)			
	Measured concentration (pg/mL)	Mean concentration found (pg/mL)	Accuracy (%)	CV (%)	Measured concentration (pg/mL)	Mean concentration found (pg/mL)	Accuracy (%)	CV (%)
LLOQ	3.03	3.10	102.31	10.12	3.03	3.18	104.95	10.46
QCL-1	9.09	9.78	107.59	4.97	9.09	10.01	110.12	2.74
QCL-2	60.63	65.08	107.34	3.87	60.63	67.01	110.53	1.55
QCM	126.30	122.38	96.89	2.18	126.30	123.45	97.74	1.39
QCH	236.82	235.64	99.50	2.64	236.82	232.39	98.13	1.28

Extraction efficiency

Liquid-Liquid extraction technique with Ethyl acetate and n-Hexane (70:30) was robust, effective, and simple. The percent recovery was assessed by comparing the peak area ratio of scopolamine in the treated samples with those in the un-treated samples, as showed by the following equation:

$$\text{Recovery (\%)} = \frac{\text{Average area ratio of drug for untreated samples}}{\text{Average area ratio of drug for treated samples}} \times 100\%$$

The recoveries of scopolamine and IS where satisfactory and reproducible. The mean overall recoveries of scopolamine and IS were 78.63% and 76.21%, respectively. The CV was less than 3.25% and 4.92% for scopolamine and IS, respectively.

Dilution integrity

The dilution integrity was assessed for samples whose concentration was as double as ULOQ concentration, which were named as QC dilution. The QC dilution samples were further diluted with interference-free plasma dilutions for determining the dilution integrity of samples. The method found to be accurate and precise up to 473.64 pg.ml^{-1}, with a dilution factor of 2.

Stability

The stability test shows that scopolamine was stable in human plasma at 25 °C for 18 h. The result of the extracted plasma samples indicates that samples were stable in the auto-sampler (25 °C) for 43 h, and they were stable after subjected to four freeze and thaw cycles. QC samples were stable when long term stability was investigated at –70 °C after 3 days. No significant loss of scopolamine can be seen during sample storage, repeated thawing and freezing conditions as shown in Table 2.

Table 2. Stability data for scopolamine in human plasma samples (n = 6).

Stability test	QC (spiked pg/mL)	Mean ± SD (pg/mL)	Accuracy / Stability (%)	Precision (%)
Bench top stability	9.09	9.85 ± 0.42	108.32	4.25
(25 °C for 18 h)	236.82	235.17 ± 2.06	99.30	0.88
Injection phase stability	9.09	8.92 ± 0.29	102.85	4.94
(2–8 °C for 43 h)	236.82	232.33 ± 4.44	100.76	1.57
Autosampler stability	9.09	9.35 ± 0.46	98.11	3.19
(25 °C for 43 h)	236.82	238.62 ± 3.74	98.11	1.91
Freeze-thaw stability	9.09	9.86 ± 0.20	108.43	2.04
(four cycles)	236.82	235.02 ± 3.07	99.24	1.30
Long-term stability	9.09	9.80 ± 0.24	107.71	2.46
(-70 °C for 3 day)	236.82	227.93 ± 3.08	96.94	1.35

Application to a pharmacokinetic study

The suggested and validated LC-MS/MS method has been successfully implemented for measuring the pharmacokinetic parameters of scopolamine in 16 healthy male volunteers. Scopolamine concentration/time profiles from all volunteers after receiving Scopolamine transdermal patch (1 mg/3 days) are presented in Fig. 3.

The results of the pharmacokinetic parameters illustrated that the average maximum plasma concentration (Cmax) of scopolamine for the twenty subjects was 95.787 ± 36.877 pg.mL^{-1} and reached at the average time of 36.03 ± 21.86 h. The other parameters were the area under the curve (AUC_{0-t} and $AUC_{0-\infty}$) for scopolamine and those were found to be 1945.255 ± 788.994 pg.h.ml^{-1} and

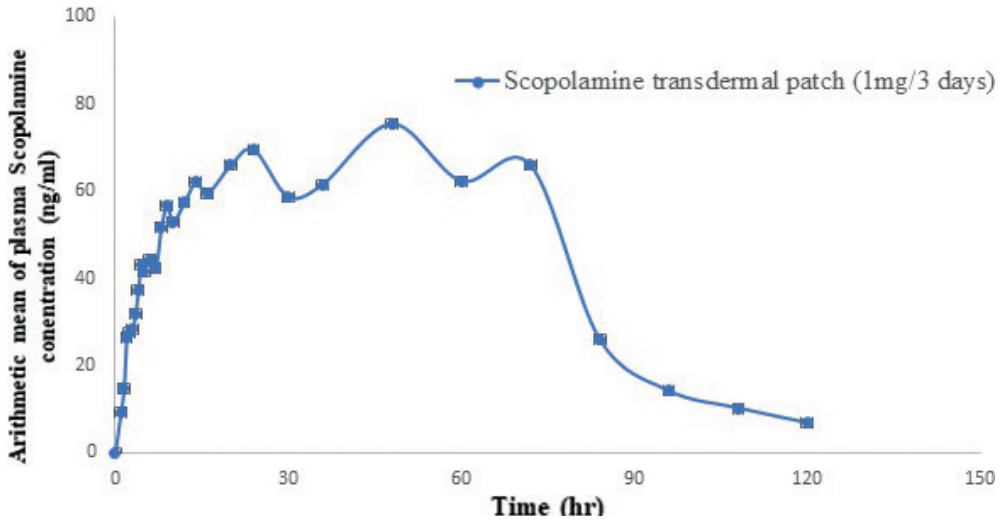

Figure 3. Mean plasma concentration following administration of a single transdermal patch of Scopolamine (1 mg/ 3 days).

5425.862 ± 1259.914 pg.h.ml^{-1} for AUC0-t and AUC0-∞, respectively as illustrated in Table 3.

Table 3. Pharmacokinetic parameters of Scopolamine transdermal patch (1 mg/3 days) (Mean ± SD).

Parameters	Scopolamine
C_{max} ± SD (ng.ml^{-1})	95.787 ± 36.877
T_{max} (h)	36.03 ± 21.86
$T_{1/2}$ (h)	6.553 ± 2.690
AUC_{0-t} (ng.h.ml^{-1})	1945.255 ± 788.994
$AUC_{0-\infty}$ (ng.h.ml^{-1})	5425.862 ± 1259.914
K_{el} (h^{-1})	0.0498 ± 0.0137

Conclusion

A rapid, simple, and sensitive LC-MS/MS for scopolamine quantification in human plasma was developed and fully validated according to FDA and EMA guideline. The used liquid-liquid extraction technique gave consistent and reproducible recoveries for Scopolamine. The method was accurate and precise for the determination of scopolamine in human plasma throughout a concentration range of 3.03–315.76 pg.ml^{-1} and should be useful for regular monitoring of drug concentrations in pharmacokinetic investigations. The method was successfully applied to determine scopolamine transdermal patches in healthy subjects and pharmacokinetic parameters were calculated.

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