Development and Validation of a precise single HPLC method for the determination of Dexamethasone in API and Pharmaceutical dosage form

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Abstract
A novel, sensitive, stability-indicating HPLC method has been developed for the quantitative estimation of dexamethasone-related impurities in both bulk drugs and pharmaceutical dosage forms. The developed method was validated as per of ICH guidelines. The chromatographic separation was achieved isocratically on RP Phenomenex Gemini C_{18} (250 mm x 4.6 mm I.D.) with particle size 5 µm and mobile phase 0.1% orthophosphoric acid: acetonitrile (60:40 v/v) was selected. It exhibited linearity over the concentration range of 2-14 µg/mL with limit of detection of 0.282 mg/mL The drug content was found to be 99.608. The present successfully validated method with excellent selectivity, linearity, sensitivity, precision and accuracy was applicable for the assay of dexamethasone in bulk drug substance and pharmaceutical dosage forms.

Key-words: Dexamethasone, Validation, Tablets, Bulk, RP-HPLC
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Introduction

Dexamethasone, a corticosteroid, is similar to a natural hormone produced by your adrenal glands. It often is used to replace this chemical when your body does not make enough of it. It relieves inflammation (swelling, heat, redness, and pain) and is used to treat certain forms of arthritis; skin, blood, kidney, eye, thyroid, and intestinal disorders (e.g., colitis); severe allergies; and asthma. Dexamethasone is also used to treat certain types of cancer. Chemically Dexamethasone is a synthetic pregnane corticosteroid and derivative of cortisol (hydrocortisone) and is also known as 1-dehydro-9α-fluoro-16α-methylhydrocortisone or as 9α-fluoro-11β,17α,21-trihydroxy-16α-methylpregna-1,4-diene-3,20-dione.

The molecular and crystal structure of dexamethasone has been determined by X-ray crystallography. It is a stereoisomer of betamethasone, the two compounds differing only in the spatial configuration of the methyl group at position 16. [1-4]

There are many reasons to validate analytical procedures. Among them are regulatory requirements, good science, and quality control requirement. The Code of Federal Regulations (CFR) 311.165c explicitly states that "accuracy, sensitivity, specificity, and reproducibility of test methods employed by the firm shall be established and documented". Of course as Scientists we would want to apply good science to demonstrate that the analytical method used had demonstrated accuracy, sensitivity, specificity and reproducibility. Finally the management methods had demonstrated uses to release its product are properly validated for its intended use so the product will be safe for human use. [5-7] The aim of present works in to develop and validate the drug using RP-HPLC method in bulk and tablet formulation.

Methodology [8-12]

Development of RP-HPLC Method for the Determination of Dexamethasone (DXM) from Bulk and Tablet

In this study, a precise, sensitive and robust gradient reversed-phase HPLC (RP-HPLC) method was developed and validated for determination of DXM in API samples. The developed method was validated based on International Conference on Harmonization (ICH) guidelines and it was proved to be accurate, precise and robust. Additionally, the limit of detection (LOD) and limit of quantification (LOQ) were also determined.

Selection of chromatographic mode

The reverse phase HPLC was selected for separation because it is convenient and rugged than other forms of the liquid chromatography and is more likely to result in a satisfactory final separation.

Selection of stationary phase

On the basis of reversed phase HPLC mode and number of carbon present in molecule (analyte) stationary phase with C_{18} bonded phase i.e. RP Phenomenex Gemini C_{18} (250 mm x 4.6 mm I.D.) with particle size 5 µm was selected.

Selection of mobile phase

The selection was made on the basis of literature survey. After assessing the solubility of drug in different solvents as well in mobile phases; acetonitrile was selected as a first choice.

Selection of detector and detection wavelength

Photo Diode Array detector was selected, as it is reliable and easy to set at the correct wavelength. From the spectra of drug, 240 nm wavelength was selected as detection wavelength.

Preparation of standard stock solution

Standard stock solution was prepared by accurately weighing and transfering 50 mg of DXM working standard in to 50 mL volumetric flask, added about 20 mL of acetonitrile and sonicated to dissolve completely, cool and diluted up to the mark with diluent. Transferred 5 mL of this solution in to 50 mL volumetric and diluted up to mark with mobile phase, which gives concentration of 100 µg/mL of DXM.

Optimization of chromatographic parameters

Optimization in HPLC is the process of finding a set of conditions that adequately separate and enable the quantification of the analytes from the endogenous material with acceptable accuracy, precision, sensitivity, specificity, cost, ease and speed.

Optimization of mobile phase strength

The mobile phase was chosen after several trials with methanol and water in various proportions. A mobile phase consisted of 0.1% orthophosphoric acid:acetonitrile (60:40 v/v) was selected to achieve symmetrical peak and sensitivity. The effects of flow rates in the ranges of 0.9 to 1.1 mL/min were examined. A flow rate of 1 mL/min gave good sensitivity, system suitability parameter and reasonable retention time; using reversed phase C_{18} column, the retention times of DXM was observed 4.54 min at 240 nm wavelength. The total time of analysis was less than 10 min.

Optimization of detection wavelength

PDA detector was used, as it is reliable and easy to set at the correct wavelength. A fixed concentration of analyte was analyzed at different wavelengths. As per the response of analyte, 240 nm wavelength was selected. A spectrum of DXM was shown in Figure.

Table 1: Final chromatographic conditions for DXM

Chromatographic mode	Chromatographic condition
• Standard solution	100 µg/mL of DXM in Acetonitrile
• HPLC System	Shimadzu HPLC system
• Pump	LC-10AT VP solvent delivery system
• Detector	SPDM-10AVP photodiode array detector
• Data processor	Class-M10 data station
• Stationary phase	Phenomenex Gemini C_{18}column (250mmx4.6mm,5µ)
• Mobile phase	0.1% orthophosphoric acid:acetonitrile (60:40 v/v)
• Detection wavelength	240 nm
• Flow rate	1 mL/min
• Sample size	20 µL
• Column temperature	27 ^{0}C

Linearity studies for DXM

From stock solution aliquots of 0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.4 mL were taken in 10 mL volumetric flasks and diluted up to the mark with methanol such that the final concentration of DXM in the range 2-14 µg/mL. Volume of 10 µL of each sample was injected with the help of syringe. All measurements were repeated five times for each concentration and calibration curve was constructed by plotting the peak area *vs* the drug concentration. The observations and calibration curve is shown in **Table and Figure.**

Application of the proposed method to bulk sample of DXM

Accurately weighed quantity 10 mg (DXM) was transferred to 100 mL volumetric flask. It was dissolved in acetonitrile by sonication and volume was adjusted to mark and sonicated. The solution was further diluted to get concentration 5 µg/mL was subjected to proposed method and amount of DXM was determined. The procedure was repeated for six times; results are shown in **Table** and chromatogram of bulk sample showed in **Figure.**

Application of proposed method to tablet formulation of DXM

To determine the content of DXM in conventional tablet (Label claim 0.5 mg Dexamethasone per Tablet) The twenty vials were weighed, their average weight determined and powder equivalent 2 mg DXM was transferred into a 10 mL volumetric flask containing 5 mL acetonitrile, sonicated for 30 min and diluted to 10 mL with mobile phase. The resulting solution was filtered, using 0.45 µm filter (Millifilter, Milford, MA). Excipients were separated by filtration. The solution was further diluted to get final concentration of 5 µg/mL was analyzed by proposed method and amount of DXM was determined. The assay procedure was repeated for six times; results are shown in **Table** and chromatogram of tablet solution in **Figure.**

Validation proposed of rp-hplc method for the determination of dexamethasone (DXN) from

bulk and formulation

The proposed method was validated as per ICH guidelines. The drug solutions were prepared as per the earlier adopted procedure given in the experiment.

Accuracy

It was done by recovery study using standard addition method at 80, 100 and 120 % level; known amount of standard DXM was added to pre-analyzed sample (5 g/mL of DXM) and analyzed by the proposed HPLC method.

Precision

Precision is the measure of how close the data values are to each other for a number of measurements under the same analytical conditions.

Repeatability

It is measured by multiple injections of a homogenous sample of 5 g/mL of DXM that indicates the performance of the HPLC instrument under chromatographic conditions.

Intra-day and Inter-day precision

Intra-day precision was determined by analyzing, the three different concentrations 6 g/mL, 8 g/mL and 10 g/mL of DXM, for three times in the same day. Day to day variability was assessed using above mentioned three concentrations analyzed on three different days, over a period of one week. This result shows reproducibility of the assay.

Robustness

To evaluate robustness few parameters were deliberately varied. The parameters include variation of flow rate, percentage of acetonitrile using 5 g/mL solution of DXM.

Sensitivity

Sensitivity of the proposed method was estimated in terms of Limit of Detection (LOD) and Limit of Quantitation (LOQ). LOD = 0.282 SD/S and LOQ = 0.896 SD/S, where SD is the residual standard deviation and Sis the slope of the line. LOD and LOQ were found to be 0.282 µg and 0.896 µg for DXM, respectively.

Specificity and Selectivity

The analytes should have no interference from other extraneous components and be well resolved from them. Specificity is a procedure to detect quantitatively the analyte in presence of component that may be expected to be present in the sample matrix, while selectivity is the procedure to detect qualitatively the analyte in presence of components that may be expected to be present in the sample matrix. The method is quite selective. There was no other interfering peak around the retention time of DXM; also the base line did not show any significant noise.

Ruggedness

From the stock solution, sample solution of DXM (5 µg/mL) was prepared and analyzed by two different analysts using similar operational and environmental conditions. Peak area was measured for same concentration solutions, six times.

System suitability test

System suitability testing is essential for the assurance of the quality performance of the chromatographic system. Earlier prepared solutions for chromatographic conditions were tested for system suitability testing.

Results and Discussion

Development of RP-HPLC Method for Determination of Dexamethasone (DXM) from Bulk and Tablets

The HPLC analysis was performed on the Phenomenex Gemini C_{18} (250 mm × 4.60 mm), 5µm particle size in isocratic mode, at 35 ^{0}C temperature using a mobile phase consisting of 0.1% orthophosphoric acid:acetonitrile (60:40 *v/v*) at a flow rate of 1.0 mL/min. Table 25 represents the different concentration of mobile phase along with retention time in minutes of DXM. The detection was carried out at 240 nm. The spectra of DXM is depicted in Figure. Table represents the final chromatographic conditions employed for the detection of DXM in bulk and in formulation. Linearity was observed in the concentration range from 2-14 µg/mL (r^2 = 0.997) as shown in the Table 27. Figure 10 depicted the Linearity of DXM with Correlation Coefficient = 0.997, Slope = 2230.68, Intercept = 246.6. The average retention time for DXM was found to be 4.54 min as shown in Figure 11. The limit of detection and quantitation of DXM was 0.282 µg and 0.896 µg, respectively. The method has been successively applied for the determination of DXM in bulk (Table 28). The method has been successively applied for the determination of DXM in tablets. There was no

interference from the excipients commonly present in the tablets. The drug content was found to be 99.608 % for DXM (Table 29). Figure 12 represents the Chromatogram of BZB Tablet solution (5 µg/mL) can be interpreted that the retention time does not affected by the excipients of the formulation.

Table 2: Optimization of mobile phase strength for estimation of DXM

Sr. No.	Mobile Phase Strength [0.1% orthophosphoric acid : acetonitrile v/v]	Flow rate [mL/min]	R_T of SLN [min]
1	50:50	1	5.10
2	60:40	1	4.54
3	70:30	1	4.98

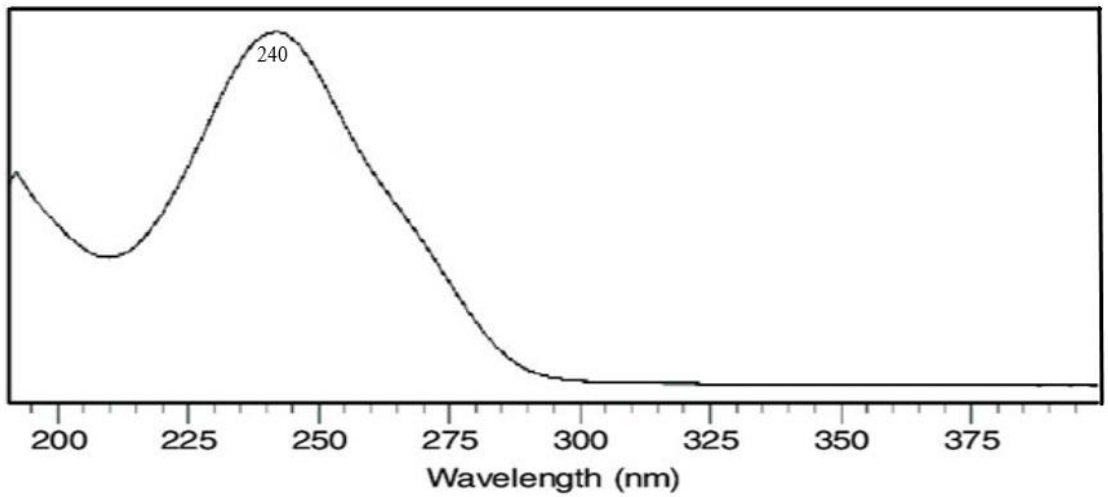


Figure 1: Spectra of DXM

Table 3: Final chromatographic conditions for DXM

Chromatographic mode	Chromatographic condition
Standard solution	100 µg/mL of DXM in acetonitrile
HPLC System	Shimadzu HPLC system
Pump	LC-10 AT VP solvent delivery system
Detector	SPD M-10AVP photo diode array detector
Data processor	Class-M 10 data station
Stationary phase	Phenomenex Gemini C_{18} column (250 mm x 4.6mm,5 µ)
Mobile phase	0.1% orthophosphoric acid:acetonitrile (60:40 v/v)
Detection wavelength	240 nm

Flow rate	1 mL/min
Sample size	20 µL
Column temperature	27 ^{0}C

Table 4: Linearity study of DXM

Sr. No.	Concentration of DXN [µg/mL]	Mean peak area [n=5]	%RSD
1	2	4578.00	1.36
2	4	9297.40	1.26
3	6	13785.47	0.98
4	8	18561.57	1.12
5	10	21875.18	1.16
6	12	26497.12	1.25
7	14	32056.42	1.32

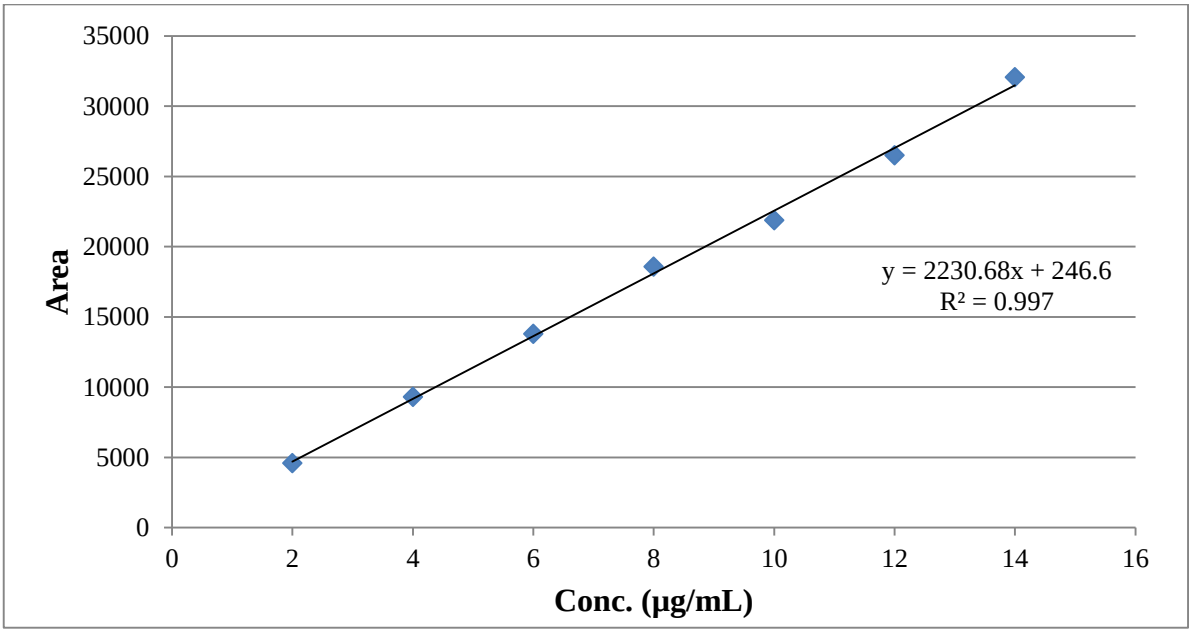


Figure 2: Linearity of DXM

Correlation Coefficient = 0.997, Slope = 2230.68, Intercept = 246.6

Table 5: Analysis of DXM in bulk sample

Component	Amount taken [µg/mL]	Amount Found [µg/mL]	Amount found [%]
DXM	5	4.962	99.24
	5	4.944	98.88
	5	5.103	102.06
	5	4.924	98.48
	5	5.042	100.84
	5	4.899	97.98
	Mean ± SD	4.979 ± 0.078	99.580 ± 1.557
	% RSD	1.563	1.563

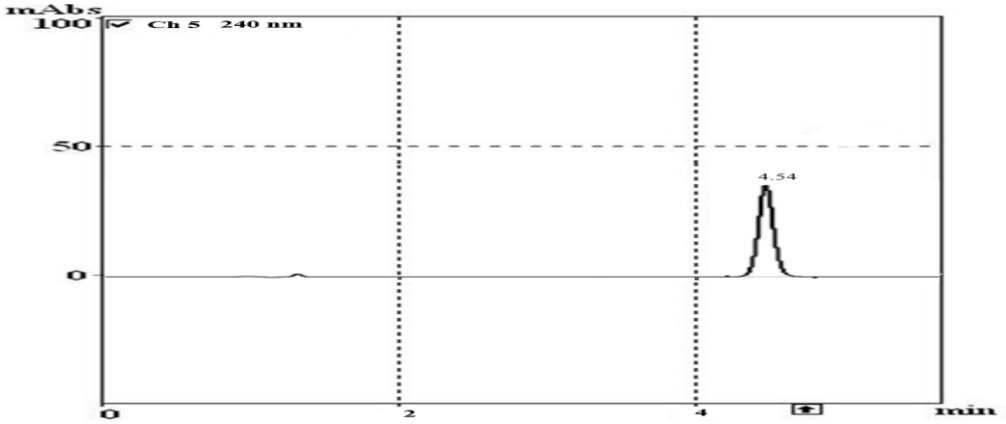


Figure 3: Chromatogram of standard DXM (5 µg/mL)

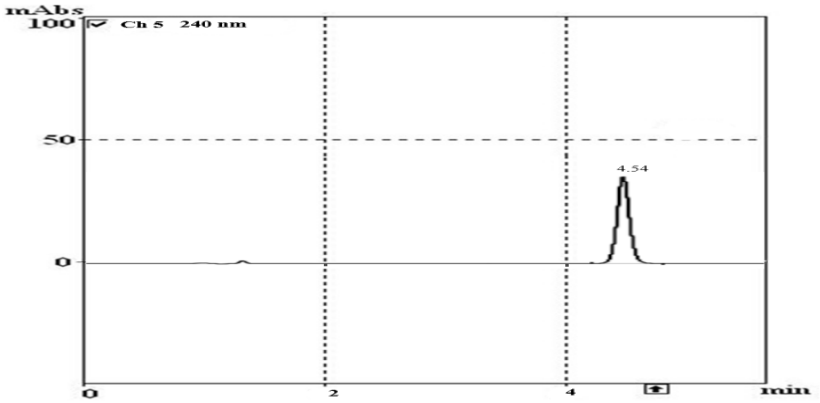
Table 6: Assay of DXM Tablet

Brand name: DECDAN (Merind Ltd., Mumbai, Maharashtra)

Batch no. BEW1464 **Mfg.: 10/2020** **Exp.: 09/2023** **Average wt = 123.058 mg**

Drugs	Label claim [mg]	Amount found [mg]	Amount found [%]
SLN	4	3.941	98.53
	4	3.968	99.20
	4	3.936	98.40
	4	4.009	100.23
	4	3.976	99.40
	4	4.068	101.70

	Mean ± SD	3.983 ± 0.049	99.575 ± 1.232
	%RSD	1.238	1.238



Validation of Proposed RP-HPLC Method for Determination of Dexamethasone (DXM) from Bulk and Tablets

Accuracy of the method was studied by the recovery studies at three different levels 80 %, 100 % and 120 % level. The results of recovery studies are presented in Table. The % recovery was found to be within the limits of the acceptance criteria with average recovery of 97.333-99.944. The mean average recovery was found to be 98.536 %. The % RSD below 2.0 shows the high precision of proposed method. According to USP (621), system suitability tests are an integral part of chromatographic methods. They are used to verify the reproducibility of the chromatographic system. To ascertain its effectiveness, system suitability tests were carried out on freshly prepared stock solutions. Results of precision studies carried out intraday and interday by using different concentrations of DXM 6, 8 and 10 g/mL and showed % RSD in range of 1.417-2.285 and 0.641-1.313 respectively. The mean percentage recovery was found to be 98.352 and 97.463 intraday and interday respectively by using different concentrations of DXM, the detailed results are summarized in table. Robustness evaluation of the HPLC method was determined by different chromatographic conditions i.e. varying in flow rate and change in concentration of mobile phase. The study was performed in triplicate. The results obtained in study are reported in table. Whereas, the table represents the results of ruggedness. The parameters obtained are shown in Table. Summary of validation parameters is shown in Table

Table 7: Results of recovery studies of DXM

Drug	Initial amount [µg/mL]	Amount added [µg/mL]	Amount recovered ± SD [µg/mL, n = 3]	% Recovery	% RSD	SEM	Variance
DXM	5	0	4.930 ± 0.056	98.600	1.129	0.032	0.003100
	5	4	3.893 ± 0.025	97.333	0.646	0.015	0.000633

5	5	4.963 ± 0.064	99.267	1.295	0.037	0.004133
5	6	5.937 ± 0.131	99.944	2.918	0.075	0.017033

Table 8: Results of repeatability for DXM

Sr. No.	Concentration [µg/mL]	Peak area
1	5	10954
2	5	12041
3	5	11623
4	5	10223
5	5	9962
6	5	10637
Mean ± SD		10906.667 ± 804.314
% RSD		7.375

Table 9: Results of precision studies of DXM (Intra-day and inter-day)

Drug	Conc. [µg/mL]	Intra-day Amount found [µg/mL]		Inter day Amount found [µg/mL]	
		Mean ± SD	% RSD [n=3]	Mean ± SD	% RSD [n=3]
DXM	2	1.973 ±0.045	2.285	1.917 ± 0.025	1.313
	4	3.887 ±0.055	1.417	3.923 ± 0.025	0.641
	6	5.953 ±0.091	01.524	5.943 ± 0.075	1.263

Table 10: Robustness evaluation of the HPLC method for DXM

Chromatographic conditions	R_T	K'	T
A: Flow rate (mL/min)			
0.90	5.83	0.91	0.93
1.00	4.54	0.96	0.91
1.10	5.34	0.85	0.94
Mean ± SD	5.237 ± 0.651	0.907 ± 0.055	0.927 ± 0.015

B: Percentage acetonitrile in mobile phase (v/v) 30 40 50	 5.52 4.58 5.69	 0.87 0.89 0.91	 0.92 0.93 0.92
Mean ± SD	5.263 ± 0.598	0.890 ± 0.020	0.923 ± 0.006

Table 11: Results of ruggedness of DXM

Analyst	Amount found of DXM [%]	% RSD [n=3]
I	98.352	1.742
II	97.463	1.072

Table 12: System suitability test of DXM

System suitability parameters	Proposed method
Retention time (T_R)	4.54
Capacity factor (K')	0.899
Theoretical plate (N)	9807
Tailing factor (T)	0.93

Table 13: Summary of validation parameters of DXM

Parameters	Observation
Linearity range (µg/mL)	2 - 14
Regression equation	y = 2230.68x – 246.6
LOD (µg)	0.282
LOQ (µg)	0.896
Recovery (%)	98.536
Precision (% RSD)	
Intra- day (n = 3)	1.417-2.285
Inter-day (n = 3)	0.641-1.313

Repeatability (n = 5)	0.74
Ruggedness (% RSD)	
Analyst I (n = 6)	1.742
Analyst II (n = 6)	1.072
Robustness	Robust
Specificity	Specific

Conclusion

From the present study it was concluded that and confirmed the suitability of the method for quantifying DXM in their Pharmaceutical dosage form. Also, the RP-HPLC method was developed and validated.

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