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A Stability Indicating RP-HPLC method for the Simultaneous Estimation of Desloratadine, Ambroxol and Pseudoephedrine in Bulk and Pharmaceutical Dosage Form

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Abstract: *A simple, rapid, precise, accurate, reproducible and stability indicating RP-HPLC method was developed and validated for the simultaneous estimation of Desloratadine (DES), Ambroxol (AMB) and Pseudoephedrine (PSE) in bulk drug and pharmaceutical formulations. The chromatographic separation was achieved using C_{18}reverse phase column (Kromasil 250mm x 4.6mm,5µm) , Buffer and Acetonitrile at the ratio 55:45 v/v as mobile phase, flow rate of 1ml/min, Column temperature maintained at 25˚C, detector wavelength 220nm and runtime 8min. Retention time was found 2.78min 3.34min and 4.75mins for Desloratadine, Ambroxol and Pseudoephedrine respectively. The developed new method was validated as per ICH guideline taking the parameters like accuracy, precision, linearity, limit of detection, limit of quantification, intermediate precision and robustness. In the linearity test correlation coefficient was found to be 0.999 for all the molecules, percentage relative standard deviation results from precision studies were 1.41, 0.53 and 1.12; mean percentage recoveries in accuracy studies were found to be 100.78%, 99.97% and 99.38% for desloratadine, ambroxol and pseudoephedrine respectively. Very low concentrations of LOD and LOQ indicate the method was highly sensitive enough. The designed validated method can be used effectively in the laboratory for regular determination of desloratadine, ambroxol and pseudoephedrine in formulation and bulk form.*
Key words: *Desloratadine, Ambroxol, Pseudoephedrine, RP-HPLC, Validation.*

I. Introduction

Desloratadine is chemically 8-chloro-6,11-dihydro-11-(4-piperidinylidene)-5*H*-benzo [5,6] cyclohepta [1,2-b] pyridine is a antihistaminic drug. Desloratadine is an non-sedative antihistaminic drug with selective H1-receptor antagonist activity. It is an active metabolite of loratadine. Desloratadine is used for relieving the symptoms of allergic rhinitis, pruritus and urticaria. Literature survey reveals that desloratadine alone and in combination with other drugs can be estimated by spectroscopic methods [1-3] and RP-HPLC [4-7]. The chemical structure of desloratadine is shown in the Figure.1

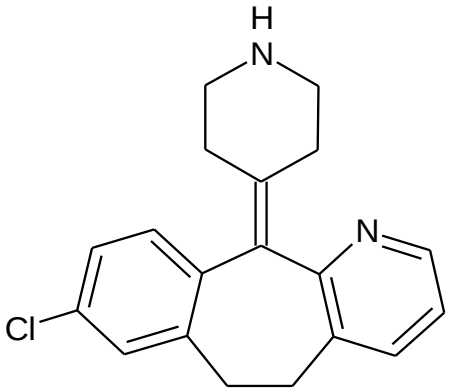

Fig.1: Structure of Desloratadine

Ambroxol is chemically known as [trans-4-[(2-amino-3,5-dibromobenzyl) amino] cyclohexanol. It is used to treat chronic inflammatory pulmonary conditions, tracheobronchitis, emphysema and asthma. Literature survey reveals that ambroxol can be estimated by Spectrophotometric method [8-12], HPLC method [13-15] either alone or in combination with other drugs. The chemical structure of ambroxol is shown in the Figure.2

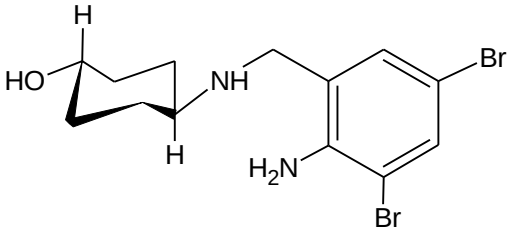

Fig.2: Structure of Ambroxol

Pseudoephedrine is chemically known as (1S,2S)-2-(methylamino)-1-phenylpropan-1-ol. It is used in the treatment of nasal congestion and in the treatment of cold and cough in combination with other drugs. Literature survey reveals that pseudoephedrine can be estimated by HPLC method [16-25] either alone or in combination with other drugs. The chemical structure of pseudoephedrine is shown in the Figure.3 Based on literature survey it was found that there is only one method has been developed for the simultaneous estimation of desloratadine, ambroxol and pseudoephedrine in combined oral tablet dosage form[26].

The main aim of the present work is to develop a new simple, rapid, precise accurate and stability indicating method for the simultaneous estimation of desloratadine, ambroxol and pseudoephedrine in combined oral tablet dosage form according to the ICH Q2 R1 guidelines [27].

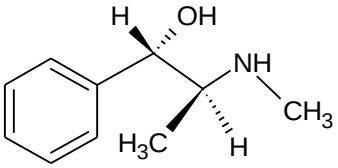

Fig.3: Structure of Pseudoephedrine

II. Materials And Methods

Instrumental and analytical conditions:

Reagents and Chemicals:

Pure samples of Desloratadine, Ambroxol and Pseudoephedrine were provided as gift samples by Spectrum Pharma Pvt.Ltd Hyderabad. The chemicals used are of HPLC grade. Acetonitrile and *o*-phosphoric acid were procured from Thermo Fischer Scientific India Pvt. Ltd. Milli Q Water was used in the buffer preparation.

Equipment:

The chromatographic separations were carried using Waters HPLC system (e2695 gradient system, Empower-2 software, model-2489) with PDA detector.

Chromatographic conditions:

Kromasil C18 column (250mm×4.6mm, 5µm particle size) was used for separation. The mobile phase consists of buffer (0.1% *o*-phosphoric acid) and acetonitrile in the ratio 55:45 v/v. The flow was adjusted to 1ml/min and total run time was set as 8min. 25˚C temperature was maintained throughout the analysis. The UV detection was achieved at 220 nm which is the Isobestic point. The injection volume was 10µL.

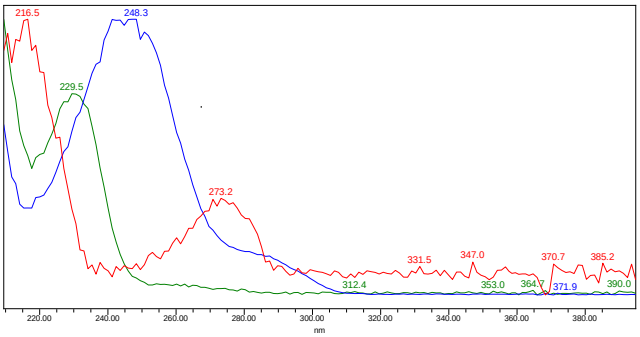

Fig.4: UV Overlain spectra of Desloratadine, Ambroxol and Pseudoephedrine showing Isobestic point

Preparation of analytical solutions:

Preparation of buffer: 1ml of Ortho-phosphoric acid is dissolved in 100ml of Milli-Q water taken in a 1000ml volumetric flask. Then made up to final volume with Milli-Q water.

Preparation of mobile phase: Buffer and acetonitrile mixed in the ratio of 55:45 v/v was used as mobile phase, sonicated and filtered through 0.22µ filter under vacuum filtration.
Diluent preparation: Acetonitrile and water mixed in the ratio of 50:50 v/v was used as diluents for sample and standard preparations.

Preparation of the standard solution:

Accurately weighed and transferred 5mg of desloratadine, 12mg of ambroxol and 6mg of pseudoephedrine into a 50ml, 10ml and 10ml volumetric flask respectively and added about 3/4th of diluent to all the flasks and sonicated for about 15min to dissolve the drugs completely, then made up to the final volume with diluent. From each of the above solutions 1ml was pippetted out and transferred into seperate10ml volumetric flasks and made up to the mark with diluent inorder to get solutions of concentration 10ppm, 120ppm, and 60 ppm for desloratadine, ambroxol and pseudoephedrine respectively.

Preparation of sample solution:

Weighed accurately 20 tablets, powdered and transferred an amount equivalent to 5mg of desloratadine, 60mg of ambroxol and 30mg of pseudoephedrine into a 50ml volumetric flask. About 30ml of diluent was added to the flask and sonicated for 30min, further the volume was made up with diluent and filtered the solution. From the filtered solution 1ml was pippeted out and transferred in to separate 10ml volumetric flasks and made up to the mark with diluents in order to get solutions of concentration 10ppm, 120ppm, and 60ppm for Desloratadine, ambroxol and pseudoephedrine respectively.

Method Development and Validation of HPLC:
The method developed was validated as per ICH guidelines with respect to following parameters such as specificity, linearity, accuracy, precision, robustness and system suitability.
Specificity:
The specificity of the method developed was carried out to determine whether there are any interference of impurities in retention time of analytical peaks. Forced degradation studies were carried out by using acidic (2N HCl), basic (2M NaOH) conditions and in presence of heat and UV light. The graph obtained for injecting the standard solution of desloratadine, ambroxol and pseudoephedrine was shown in the figure 5.

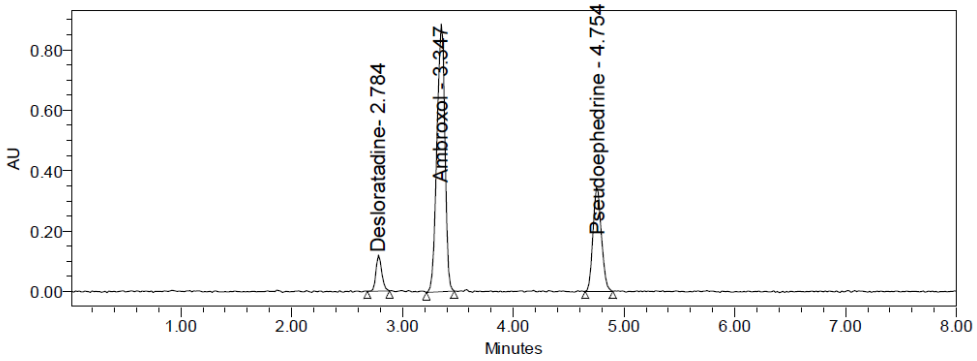

Fig.5: HPLC chromatogram of the developed method

Linearity:
The linearity of the method was established by injecting six different concentration levels of desloratadine (2.5ppm to 15ppm), ambroxol (30ppm to 180ppm) and pseudoephedrine (15ppm to 90ppm) solutions in to the HPLC system and peak areas were recorded. The results were shown in table no:1

Table No. 1: Linearity results for desloratadine, ambroxol and pseudoephedrine

Parameter	Regression equation parameters		
	Desloratadine	Ambroxol	Pseudoephedrine
Linearity range (ppm)	2.5-15	30-180	15-90
Correlation co-efficient	0.999	0.999	0.999
Slope	40438	34355	29469
Y-intercept	9700	18526	45836
LOD(ppm)	0.22	0.06	0.02
LOQ(ppm)	0.67	0.18	0.05

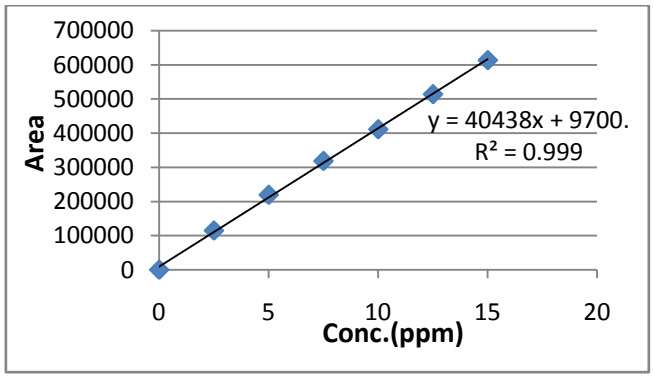

Fig .5. Showing linearity for Desloratadine

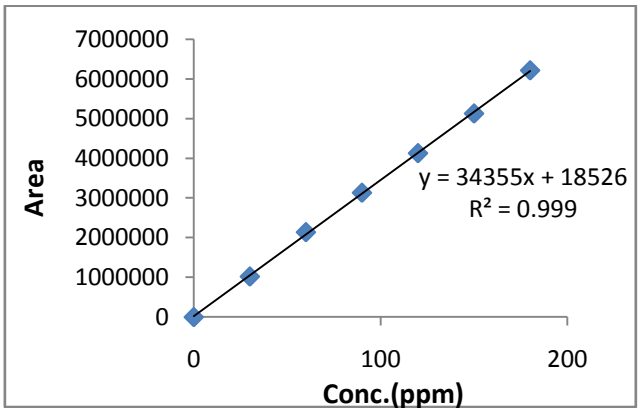

Fig.6. Showing linearity for ambroxol

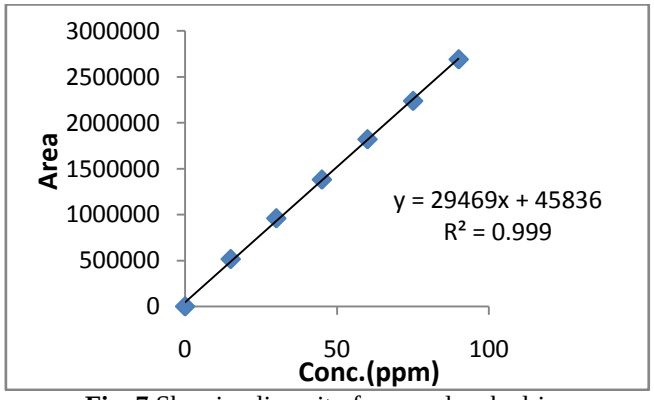

Fig .7. Showing linearity for pseudoephedrine

Accuracy: Accuracy of the developed method was determined standard addition method (n=average of 3 analyses). In this method known amounts of desloratadine, ambroxol and pseudoephedrine were supplemented to the previously analysed sample solution and then experimental and true values were compared. Three levels were made corresponding to 50%, 100% and 150% of the nominal analytical concentration. The results were shown in table no:2

Table 2: Accuracy results

Drugs	**Spiked concentration (ppm)**		**Standard addition measured concentration (ppm) ± SD; RSD (%)**
Desloratadine	5	50%	100.38±0.4; 0.00
	10	100%	100.78±1.1; 0.01
	15	150%	99.61±0.5; 0.01
Ambroxol	60	50%	100.11±0.4; 0.00
	120	100%	99.97±0.3; 0.00
	180	150%	99.23±0.7; 0.01
Pseudoephedrine	30	50%	99.79±0.6; 0.01
	60	100%	99.38±0.8; 0.01
	90	150%	100.59±0.7; 0.01

Precision:

Precision expresses the closeness of agreement between the series of measurements obtained from multiple sampling of same homogeneous samples under the prescribed conditions. Method precision was determined both in terms of repeatability and intermediate precision/ruggedness (show the degree of reproducibility of test results obtained by analyzing the sample under variety of normal test conditions such as analyst to analyst variation and instrument to instrument variation). The retention time and peak area was determined and expressed as mean and %RSD calculated from the data obtained, which are found to be within the specified limits. The system precision values are shown in table no: 3, 4 and 5.

Table No. 3: System Precision values for desloratadine

S.No	Peak Name	Rt (min)	Area	USP Plate Count	USP Tailing
1	DES _Injection -1	2.784	411061	12753	1.13
2	DES _Injection -2	2.785	410387	14590	1.29
3	DES _Injection -3	2.785	413210	14414	1.09
4	DES _Injection -4	2.785	415743	14215	1.14
5	DES _Injection -5	2.786	425931	13473	1.25
6	DES _Injection -6	2.786	411491	14045	1.2
Mean		2.79	414637		
Std. Dev.		0.00	5857.7		
% RSD		0.00	1.413		

Table No. 4: System Precision values for ambroxol

S.No	Peak Name	Rt (min)	Area	USP Plate count	USP tailing
1	AMB _Injection -1	3.347	4411340	9838	0.93
2	AMB _Injection -2	3.348	4393570	9559	0.89
3	AMB _Injection -3	3.349	4383591	6542	0.88
4	AMB _Injection -4	3.349	4451179	9562	0.93
5	AMB _Injection -5	3.35	4420398	11205	0.89
6	AMB _Injection -6	3.351	4411985	13758	0.88
Mean		3.349	4412011		
Std. Dev.		0.00	23463.6		
% RSD		0.00	0.532		

Table No. 5: System Precision values for pseudoephedrine

S.No	Peak Name	Rt (min)	Area	USP Plate count	USP tailing
1	PSE _Injection -1	4.754	1829464	18228	1.1
2	PSE _Injection -2	4.754	1832454	17420	1.15
3	PSE _Injection -3	4.755	1829404	17472	1.11
4	PSE _Injection -4	4.755	1815811	18034	1.13
5	PSE _Injection -5	4.755	1863755	17112	1.15
6	PSE _Injection -6	4.765	1802504	17217	1.11
Mean		4.756	1828899		
Std. Dev.		0.00	20475.1		
% RSD		0.00	1.12		

Robustness:

The robustness of newly developed method was assessed by evaluating the influence of small deliberate variations in procedure variables such as flow rate (±5%) and change in wave length (±5nm). The robustness was evaluated by changing the flow rate from 0.8ml/min to 1.2ml/min and the method is found robust only in low flow rate conditions and even by changing the mobile phase ±5%. The results were shown in table no:6

Table 6: Results of robustness study

Chromatographic conditions	**Average area**			**Rt(min)**		
	DES	**AMB**	**PSE**	**DES**	**AMB**	**PSE**
Buffer: Acetonitrile 60:40(v/v)	374832	3867183	1587499	2.739	3.265	4.413
Buffer: Acetonitrile 55:45(v/v)	414637	4412011	1828899	2.784	3.347	4.754
Buffer: Acetonitrile 50: 50(v/v)	391711	3806944	1559990	2.771	3.364	5.286
Flow rate (0.8 mL/min)	450951	4562617	1886744	3.068	3.700	5.235
Flow rate (1.0 mL/min)	414637	4412011	1828899	2.784	3.347	4.754
Flow rate (1.2 mL/min)	388104	3451782	1425384	2.502	3.011	4.274
Temperature 28°C	389016	3876219	1589844	2.756	3.314	4.704
Temperature 30°C	414637	4412011	1828899	2.784	3.347	4.754
Temperature 32°C	396004	3966217	1649582	2.764	3.323	4.714

System Suitability:
System suitability tests were carried out on freshly prepared standard stock solutions of desloratadine, ambroxol and pseudoephedrine, it was calculated by injecting standards in six replicates and the values were recorded and are within the limits (%RSD <2) as shown in table no.7

Table No.7: System suitability Parameters

Parameter	Results			Limits
	Desloratadine	Ambroxol	Pseudoephedrine	
RSD of peak area	1.41	0.53	1.12	<2.0
RSD of retention time	0.00	0.00	0.00	<1.0
USP tailing factor (T)	1.29	0.93	1.15	T < 2
USP plate count (N)	12753	6542	17112	>2000
USP resolution (R)	--	4.5	9.3	> 2

Forced Degradation studies:
Oxidation:
To 1ml of stock solution of desloratidine, ambroxol and pseudoephedrine, 1ml of 20% hydrogen peroxide (H_2O_2) was added separately. The solutions were kept for 30 min at 60^0C. For HPLC study, the resultant solution was diluted to obtain 10ppm, 120ppm, and 60ppm solution and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of sample.
Acid Degradation:
To 1ml of stock solution desloratidine, ambroxol and pseudoephedrine 1ml of 2N Hydrochloric acid was added and refluxed for 30mins at 60^0C. The resultant solution was diluted to obtain 10ppm, 120ppm, and 60ppm solution and 10 µl solutions were injected into the system and the chromatograms were recorded to assess the stability of sample.
Alkali Degradation:
To 1ml of stock solution desloratidine, ambroxol and pseudoephedrine, 1ml of 2N sodium hydroxide was added and refluxed for 30mins at 60^0C. The resultant solution was diluted to obtain 10ppm, 120ppm, and 60ppm solution and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of sample.
Dry Heat Degradation:
The standard drugs desloratidine, ambroxol and pseudoephedrine were placed in oven at 105°C for 6h and used for preparing 10ppm, 120ppm, and 60ppm solutions. 10µl were injected into the system and the chromatograms were recorded to assess the stability of the sample**.**
Photo Stability studies:
The photochemical stability of the drug was also studied by exposing the stock solution to UV Light by keeping the flasks in UV Chamber for 7days or 200 Watt hours/m^2 in photo stability chamber. For HPLC study, the resultant solution was diluted to obtain 10ppm, 120ppm and 60ppm solutions and 10 µl were injected into the system and the chromatograms were recorded to assess the stability of sample.
Neutral Degradation Studies:
Stress testing under neutral conditions was studied by refluxing the drug in water for 6h at a temperature of 60ºC. For HPLC study, the resultant solution was diluted to 10ppm, 120ppm, and 60ppm solution and 10µl were injected into the system and the chromatograms were recorded to assess the stability of the sample**.**

Table 8: Results of Forced degradation study

Stress conditions	% Assay of active ingredients					
	DES	% Degradation	AMB	% Degradation	PSE	% Degradation
Acid, 2N HCl,	95.61	4.39	96.31	3.69	95.72	4.28
Base, 2N NaOH	96.74	3.26	99.07	0.93	96.56	3.44
H_2O_2 (20%,v/v)	98.90	1.1	98.44	1.56	97.74	2.26
Dry heat (80 ºC)	99.20	0.8	99.51	0.49	99.05	0.95
UV (365 nm)	99.40	0.6	99.48	0.52	99.09	0.91
Water	99.57	0.43	99.56	0.44	99.46	0.54

III. Results And Discussions

The present investigation reported is a new RP-HPLC method development and validation of simultaneous estimation of desloratadine, ambroxol and pseudoephedrine. The method development was proceeding with wavelength selection. The optimized wavelength was 220nm. In order to get the optimized RP-HPLC method various mobile phases and columns were used. From several trials final method is optimized with the following conditions:

The mobile phase consists of buffer and acetonitrile in the ratio of 55:45 v/v and the column used was Kromasil ODS C18 column (250mm×4.6mm,5µm particle size). The flow rate was adjusted to 1ml/min. The

instrument was operated at an ambient temperature. The UV detection was achieved at 220nm and purity analysis was performed over a wavelength range of 200-400nm.The injection volume was 10µL.The specificity of the method was to determine whether there are any interference of any impurities (the presence of components may be unexpected to present) in retention time of analytical peak. The calibration curve obtained by plotting peak area versus concentration was linear and the correlation coefficient was found to be 0.999, 0.999 and 0.999 for desloratadine, ambroxol and pseudoephedrine respectively. The linearity graphs were shown in Figure 5, Figure 6 and Figure 7.

The precision of the method was ascertained from determinations of peak areas 6 replicates of sample solution. The % relative standard deviation for system precision of desloratadine, ambroxol and pseudoephedrine was found to be 1.41, 0.53 and 1.12 respectively and presented in table no: 3,4 and 5. The % relative standard deviation for the method precision of desloratadine, ambroxol and pseudoephedrine was found to be 1.10, 0.97 and 0.54. The accuracy study was performed in 50%, 100% and 150%. The percentage recovery was determined for desloratadine, ambroxol and pseudoephedrine was found to be 100.3, 99.8 and 99.9% the results were presented in table no: 2**.** The robustness were carried out with minor but deliberate changes in parameters i.e., detection wavelength, column temperature and flow rate were presented in table no: 6. Theoretical plate values and tailing factor for desloratadine, ambroxol and pseudoephedrine were within the acceptance limits i.e theoretical plates more than 2000 and tailing factor not more than 2. The results were presented in table no: 7. Forced degradation studies were performed to establish the stability indicating property and specificity of the proposed method. Degradation studies were carried out under conditions of acid hydrolysis, base hydrolysis, thermal, oxidation, and photolysis and the drug substances were observed high degradation in acid (2N HCl) comparative remaining in all conditions. Thermal degradation conditions were performed by heating the drug sample at 80˚C. Acid and base hydrolysis showed slight (3-4%) degradation, very slight degradation observed in oxidation, thermal, photolytic hydrolysis. The results of forced degradation studies were given in table no: 8.

IV. Conclusion

The newly developed method was found to be simple, rapid, precise, accurate, reproducible and stability indicating for determination of desloratadine, ambroxol and pseudoephedrine in pharmaceutical tablet dosage form. The newly developed method was validated according to ICH guidelines for parameters such as specificity, linearity, accuracy, precision, robustness and system suitability, values were found to be within limits. The validation study indicates that method can be considered suitable for carrying out quality control and routine analysis of desloratadine, ambroxol and pseudoephedrine in bulk drug and pharmaceutical tablet dosage form.

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