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Development of Validated Specific Stability Indicating HPTLC Method for Simultaneous Estimation of Enalapril Maleate and Losartan Potassium in its Combined Dosage Form

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The objective of current study was to develop a validated, specific stability indicating normal-phase high performance thin layer chromatographic method for simultaneous estimation of enalapril maleate and losartan potassium in their combined dosage form. The forced degradation studies were performed on pure enalapril maleate and losartan potassium and also on their combined dosage form using acid, base, neutral, oxidation, thermal and photo stress to show the stability indicating capability of the developed method. Significant degradation products of enalapril maleate were observed in acidic, basic and thermal stress whereas losartan potassium were degrade in photo stress. No degradation products were obtained after neutral and oxidation stress conditions. The chromatographic method was optimized using samples generated in forced degradation studies. Good separation between the peaks corresponding to the active pharmaceutical ingredients, enalapril maleate and losartan potassium and degradation product from the analyte were achieved on silica gel $60F_{254}$ TLC plate using toluene: ethyl acetate: methanol: acetic acid in ratio 5:2.5:2.5:0.1 (v/v) as mobile phase. Densitometric quantification was performed at 213 nm by reflectance scanning. The R_F values of enalapril maleate and losartan potassium were 0.49 ± 0.03 and 0.66 ± 0.03, respectively. Validation of the developed method was conducted as per ICH requirements. Response were a linear function of concentration of enalapril maleate over the range 50-400 ng/band by peak area with correlation coefficient 0.99523 and losartan potassium over the range 250-2000 ng/band by peak area with correlation coefficient 0.99030. The limit of detection of enalapril maleate was 4.58 ng/band for peak area and the limit of detection of losartan potassium was 7.26 ng/band for peak area. Results from analysis of a commercial tablet formulation were 100.17 ± 1.170 % and 98.97 ± 0.6243 % for enalapril maleate and losartan potassium, respectively. Recoveries were 98.69 ± 1.3373 % and 100.15 ± 0.5016 % for enalapril maleate and losartan potassium, respectively.

Keywords: HPTLC, Enalapril maleate, Losartan potassium, Degradant, Validation.

INTRODUCTION

Enalapril maleate is chemically (1-{N-[(s)-1-carboxyl-3-phenylpropyl]-L-alanyl-}-L-proline-1-ethyl ester maleate) (Fig. 1). It is an angiotensin-converting-enzyme inhibitor used in the treatment of hypertension, diabeticnephropathy and some types of chronic heart failure. Losartan potassium is chemically 2-butyl-4-chloro-1-[*p*-(*o*-1H-tetrazol-5-ylphenyl)-benzyl]imidazole-5-methanol monopotassium salt (Fig. 2). It is an angiotensin II receptor antagonist drug used mainly to treat high blood pressure (hypertension) [1-4].

Literature survey revealed estimation of enalapril maleate and losartan potassium by UV spectroscopy in tablet alone [5-8] and in combination with other drug [9], HPTLC in alone [10,11] and in combination with other drugs [12-14] and HPLC in alone [15-19] and in combination with other drug [20,21] has been reported. The reported HPTLC method is only suitable for simultaneous estimation of enalapril maleate and losartan potassium in the bulk drug and dosage form in presence of their degradation products.

EXPERIMENTAL

Pharmaceutical grade enalapril maleate and losartan potassium were procured as a gift samples from Cadila Pharmaceuticals Ltd., Ahmedabad (India), ENVAS-RB 25 a tablet formulation, obtained commercially. Toluene, ethyl acetate, acetic acid, methanol, hydrochloric acid, sodium hydroxide and hydrogen peroxide 30 % of analytical grade were used throughout the work.

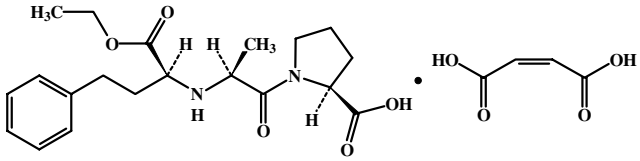

Fig. 1. Chemical structure of enalapril maleate

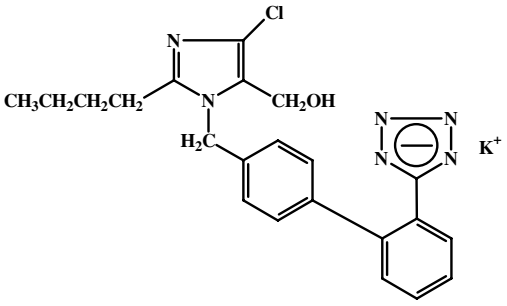

Fig. 2. Chemical structure of losartan potassium

Preparation of standard solution: For enalapril maleate, an accurately weighed 4 mg of enalapril maleate was transferred to 10 mL volumetric flask and dissolved in 5 mL of methanol. The volume was completed to 10 mL with methanol. Resulting solution of 1 mL was pipetted in 10 mL volumetric flask and the volume was made up to 10 mL with methanol to furnish a solution of concentration 40 µg/mL of enalapril maleate.

For losartan potassium, an accurately weighed 20 mg of losartan potassium was transferred to 10 mL volumetric flask and dissolved in 5 mL of methanol. The volume was completed to 10 mL with methanol. Resulting solution of 1 mL was pipetted in 10 mL volumetric flask and the volume was made up to 10 mL with methanol to furnish a solution of concentration 200 µg/mL of losartan potassium.

For the working mixed standard solution, an accurately weighed 4 mg of enalapril maleate and 20 mg of losartan potassium were transferred to 10 mL volumetric flask and dissolved in 5 mL of methanol. The volume was completed to 10 mL with methanol. Resulting solution of 1 mL was pipetted in 10 mL volumetric flask and the volume was made up to 10 mL with methanol to furnish a solution of concentration 40 and 200 µg/mL of enalapril maleate and losartan potassium, respectively.

Preparation of sample solution: Twenty tablets were weighed and finely powdered. An accurately weighed amount of powder equivalent to 4 mg of enalapril maleate and 20 mg of losartan potassium was transferred into a 10 mL volumetric flask. Then 5 mL of methanol was added in it. The flask contents were sonicated for 10 min to make the contents homogeneous. This solution was then diluted up to the mark with methanol. The resultant solution was filtered through Whatman grade I filter paper. 1 mL of filtrate was transferred to 10 mL volumetric flask and then volume was made up to the mark with methanol to furnish a sample solution containing 40 µg/mL of enalapril maleate and 200 µg/mL of losartan potassium.

Six replicate of tablet powder equivalent to 4.0 mg of enalapril maleate and 20 mg of losartan potassium were transferred into six 10 mL volumetric flask and homogenous sample solutions were prepared in a similar manner.

Chromatography: Chromatography was performed on 10 cm × 10 cm HPTLC plates coated with silica gel 60 F_{254}. Before use plates were washed with AR-grade methanol and activated at 115 °C for 0.5 h. Samples (5 µL) were applied to the plates as bands 4 mm wide and 3 mm apart by use of a CAMAG Linomat IV automatic sample applicator equipped with a Hamilton syringe. The application rate was 5 s/µL.

Linear ascending development to a distance of 80 mm was performed in a 10 cm × 10 cm CAMAG twin-trough chamber using toluene: ethyl acetate: methanol: acetic acid in ratio 5:2.5:2.5:0.1 (v/v) as mobile phase. Before the insertion of the plate, the chamber was saturated with mobile phase vapour for 10 min at room temperature and after the insertion of plate again saturated for 10 min. After development the plate was removed and dried with hot air drier. Densitometric scanning was performed at 213 nm with a CAMAG TLC Scanner III in reflectance-absorbance mode controlled by CATS 4 software (version 1.4.1; CAMAG) resident in the system. The slit dimensions were 3.00 mm × 0.45 mm and the scanning speed 20 mm/s. The radiation source was a deuterium lamp emitting continuous UV radiation between 190 and 360 nm. The amounts of the compounds chromatographed were determined from the intensity of diffusely reflected light.

Method validation

Stress studies and specificity: Stress testing of drug substances can help to identify the likely degradation products, which can, in turn, help to establish the degradation pathways and the intrinsic stability of the drug substances. Specificity is the ability of the method to measure the responses of the analyte in the presence of its related substances. All stress degradation studies were performed at initial drug concentrations of 0.4 and 2.0 mg/mL for enalapril maleate and losartan potassium, respectively. Acid hydrolysis was performed in 0.1N HCl at 80 °C for 12 h. The study in basic solution was conducted in 0.1 N NaOH on initial sample at room temperature. Neutral hydrolysis was performed at 80 °C for 12 h. Oxidation studies were conducted at room temperature in 30 % hydrogen peroxide for 24 h. For photo degradation studies, the drug sample was exposed to sun light for 30 days. The drug sample was exposed to dry heat at 70 °C for 30 days. Samples were withdrawn at appropriate times and subjected to HPTLC analysis after suitable dilution to evaluate the ability of the proposed method to separate enalapril maleate and losartan potassium from their degradation products. Assessment of the mass balance in the degraded samples was conducted to confirm that the amount of degraded product detected in stressed samples matched with the amount present before the stress was applied. Quantitative determination of enalapril maleate and losartan potassium was conducted in all stressed samples against qualified working standards, which is tabulated in Table-1.

Limit of detection (LOD) and limit of quantitation (LOQ): The LOD is the lowest analyte concentration that can be detected. LOQ is the lowest analyte concentration that can be quantified with acceptable accuracy and precision. The limits of detection and limit of quantification were calculated from the standard deviation of the response and the slope of calibration plot. LOD and LOQ were established, in accordance with ICH definitions [22], by use of the equations LOD = 3.3 σ/S and LOQ = 10 σ/S, where σ is the standard deviation of the regression line and S is the slope of the calibration plot.

Linearity: Linearity test solutions of enalapril maleate and losartan potassium were prepared at concentration levels of 10 to 80 µg/mL and 50 to 400 µg/mL, respectively. Linearity test solutions were prepared by diluting the stock solution to the required concentrations. Linearity was established by least-

TABLE-1
DEGRADATION STUDY

Formulation ENVAS-RB 25	Normal	Acid	Alkali	Neutral	Oxide	Heat	Photo
Enalapril maleate (%)	99.98	97.98	97.12	101.25	100.49	100.78	101.04
Losartan potassium (%)	99.76	100.25	99.98	100.36	99.23	99.79	94.45

squares linear regression analysis of the calibration data. Peak areas were plotted against the respective concentrations and linear regression analysis performed on the resulting curves.

Precision: The system precision was evaluated by measuring area of six bands of qualified working standard for enalapril maleate and losartan potassium and calculating the percentage of relative standard deviation (RSD). The assay method precision was evaluated by conducting six independent assays of test samples of enalapril maleate and losartan potassium against qualified working standards and calculating the percentage of relative standard deviation. The intermediate precision of the method was also verified using different analysts and different days.

Accuracy: The accuracy of an analytical procedure expresses the closeness of agreement between the value, which is accepted either as a conventional true value or an accepted reference value and the value found. The accuracy of the assay method was evaluated in triplicate at three concentration levels, *i.e.*, 80, 100 and 120 % of the label claim. Standard addition and recovery experiments were conducted to determine the accuracy of enalapril maleate and losartan potassium for the quantification of drug in the samples.

Robustness: To evaluate the robustness of the developed method, the chromatographic conditions were deliberately altered and the resolution between enalapril maleate and losartan potassium was evaluated. To study the effect of wavelength on the estimation, the wavelength was altered by ± 2 nm, *i.e.*, 211 and 215 nm from the actual wavelength, 213 nm. To study the effect of mobile phase composition on estimation, methanol composition was altered by ± 0.2 mL *i.e.*, 2.3 and 2.7 mL from the actual volume, 2.5 mL. To study the effect of saturation time on estimation, saturation time was altered by ± 5 min *i.e.*, 15 and 25 mL from the actual time, 20 min.

RESULTS AND DISCUSSION

HPTLC optimization: Initially, pure drugs solution was chromatographed using single solvents to ascertain the movement of the drug. Use of toluene: ethyl acetate: methanol: acetic acid 5:2.5:2.5:0.1 (v/v) as mobile phase gives well resolved peaks of drugs and separation of degradation products from drugs as well. The R_F value of enalapril maleate and losartan potassium were found to be 0.49 ± 0.03 and 0.66 ± 0.03, respectively. Maleic acid (MA) due to enalapril maleate was found at R_F value 0.32 ± 0.03 which was confirmed by applying separate band of 40 ppm solution of sodium maleate on same plate. Typical HPTLC densitogram (213 nm) was obtained from standard solution is shown in Fig. 3.

Then samples obtained from forced degradation were then chromatographed with the same mobile phase and it was found that densitogram obtained after acidic hydrolysis gave degradation product of enalapril maleate at R_F value 0.74 ± 0.03 (EDP-I), alkaline hydrolysis gave degradation product of enalapril maleate at R_F values 0.38 ± 0.03 (EDP-II), heat

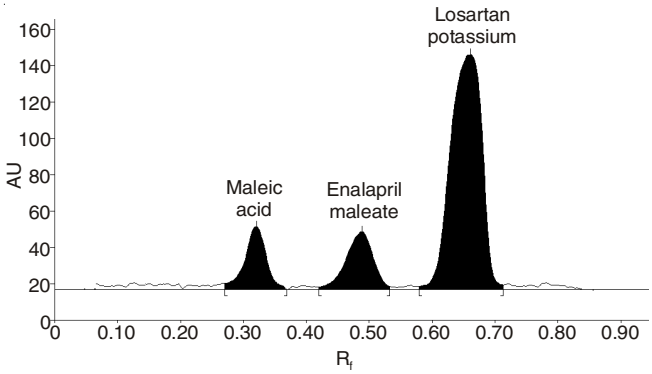

Fig. 3. Densitogram of enalapril maleate and losartan potassium combination

stress condition gave degradation product of enalapril maleate at R_F values 0.74 ± 0.03 (EDP-III), photo stress condition gave two degradation products of losartan potassium at R_F values 0.39 ± 0.03 (LDP-I) and 0.78 ± 0.03 (LDP-II) (Fig. 4). No degradation products of enalapril maleate and losartan potassium were obtained after neutral and oxidation stress condition. Toluene:ethyl acetate:methanol:acetic acid 5:2.5:2.5:0.1 (v/v) was therefore used as mobile phase and resulted in sharp, well defined, symmetrical peaks with no fronting when scanning was performed at 213 nm. The assay of enalapril maleate and losartan potassium was unaffected by the presence of degradation products, which confirms that the HPTLC method is stability-indicating. There was no interference from common excipients present in the tablet. Linear ascending development to a distance of 80 mm was performed in a 10 cm × 10 cm CAMAG twin-trough chamber. Before the insertion of the plate, the chamber was saturated with mobile phase vapour for 10 min at room temperature and after the insertion of plate again saturated for 10 min. After development the plate was removed and dried with hot air drier. Densitometric scanning was performed at 213 nm with a CAMAG TLC Scanner III in reflectance-absorbance mode controlled by CATS 4 software (version 1.4.1; CAMAG) resident in the system. The slit dimensions were 3.00 mm × 0.45 mm and the scanning speed 20 mm/s. The radiation source was a deuterium lamp emitting continuous UV radiation between 190 and 360 nm. The amounts of the compounds chromatographed were determined from the intensity of diffusely reflected light.

Limit of detection and limit of quantification: The LOD of enalapril maleate and losartan potassium were 4.58 and 7.26 ng per band for peak area, respectively. The LOQ of enalapril maleate and losartan potassium were 13.87 and 22.02 ng per band for peak area, respectively.

Linearity: Linearity was established by least-squares linear regression analysis of the calibration data. Calibration plots were linear over the concentration range 50-400 ng/band by area for enalapril maleate and 250-2000 ng/band by area for losartan potassium. Peak areas were plotted against the respective concentrations and linear regression analysis performed on the resulting curves. Equation for the calibration plots

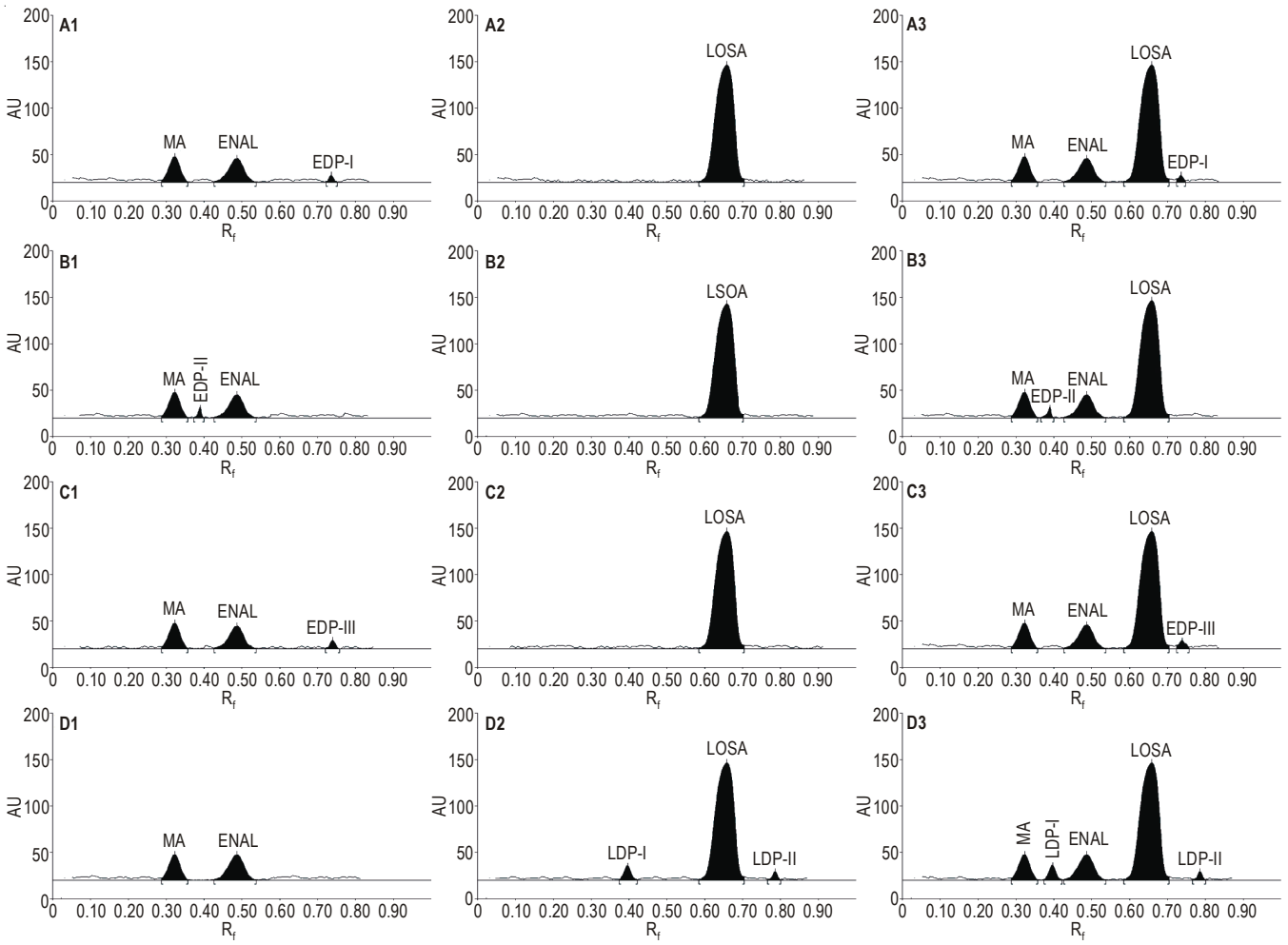

Fig. 4. Results from forced degradation of (1) enalapril maleate, (2) losartan potassium and (3) tablet powder in (A) 0.1 N HCl, 12 h at 80 °C (B) 0.1 N NaOH, initial at room temperature (C) Thermal, 30 days at 70 °C (D) Sunlight, 30 days

of enalapril maleate was Y= 429.092 + 3.516*X, for peak area. Correlation coefficient was 0.99523 for peak area. Equation for the calibration plots of losartan potassium was Y= 3500.338 + 1.680*X, for peak area. Correlation coefficient was 0.99030 for peak area.

Precision: The percentage RSD of system, method and intermediate precision study was well within ± 2.0 %.

Results of system, method and intermediate precision are summarized in Table-2.

Accuracy: The percentage recoveries were 98.69 ± 1.3373 % and 100.15 ± 0.5016 % by peak area for enalapril maleate and losartan potassium, respectively. The RSD value was found to be less than 2 % (Table-3).

Robustness: Results of robustness studies are summarized in Table-4.

The method enables simple, specific and accurate analysis of enalapril maleate and losartan potassium and its degradation products in combined dosage form. This method was validated as per ICH guidelines. The method can therefore be used for routine quality-control analysis of enalapril maleate and losartan potassium in combined dosage forms.

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TABLE-2
SYSTEM, METHOD AND INTERMEDIATE PRECISION DATA

Validation parameters		Enalapril maleate			Losartan potassium		
		Mean	SD (±)	RSD (%)	Mean	SD (±)	RSD (%)
System precision[a]		1121.56	8.9546	0.7984	5331.03	39.9854	0.7500
Method precision[a]		100.28 %	1.1740	1.1706	98.97 %	0.6243	0.6307
Intermediate precision	Intra-day[b]	98.58 %	0.7494	0.7601	99.45 %	0.3404	0.3423
	Inter-day[b]	97.44 %	0.7817	0.8022	99.19 %	0.1650	0.1663
	Different analyst[b]	100.83 %	0.4521	0.4484	99.51 %	0.3376	0.3376

[a]Mean from six analyses (n = 6); [b]Mean from 3 analyses (n = 3)
n = Number of samples, SD = standard deviation; RSD = relative standard deviation

TABLE-3
ACCURACY OF DATA

	Level (%)	Weight of sample (mg)	Amount of standard added (mg)	Calculated weight of drug (mg)	Recovery (%)
Enalapril maleate	80	84.5	1.6	1.57	98.12
		84.1	1.6	1.59	99.37
		83.9	1.6	1.55	96.87
	100	84.1	2.0	1.96	98.00
		84.0	2.0	1.99	99.50
		84.4	2.0	2.02	101.00
	120	84.7	2.4	2.40	100.00
		84.3	2.4	2.35	97.91
		84.2	2.4	2.34	97.50
				Mean ± SD	98.69 ± 1.3373
				RSD (%)	1.3550
Losartan potassium	80	84.5	8.1	8.09	99.87
		84.1	8.0	8.02	100.25
		83.9	7.9	8.00	101.26
	100	84.1	10.0	9.95	99.50
		84.0	10.0	10.00	100.00
		84.4	9.9	9.90	100.00
	120	84.7	11.9	11.96	100.50
		84.3	12.1	12.02	100.16
		84.2	12.0	11.98	99.83
				Mean ± SD	100.15 ± 0.5016
				RSD (%)	0.5008

TABLE-4
ROBUSTNESS

Condition		Enalapril maleate (By peak area*)		Losartan potassium (By peak area*)	
		Amount estimated (%) ± SD	RSD (%)	Amount estimated (%) ± SD	RSD (%)
Change in wavelength (213 ± 2 nm)	211 nm	97.28 ± 0.5543	0.5698	100.16 ± 0.0901	0.0900
	215 nm	98.21 ± 0.5631	0.5733	100.08 ± 0.1418	0.1417
Change in mobile phase composition (± 0.2 mL)	Toluene:ethyl acetate:methanol:acetic acid = 5.0: 2.5: 2.3: 0.1 (v/v)	99.07 ± 0.6155	0.6213	100.81 ± 0.1890	0.1875
	Toluene:ethyl acetate:methanol:acetic acid = 5.0: 2.5: 2.7: 0.1 (v/v)	99.00 ± 0.4800	0.4848	99.51 ± 0.1908	0.1918
Change in saturation time (20 ± 5min)	15 min	99.55 ± 0.6352	0.6381	99.37 ± 0.2055	0.2068
	25 min	99.24 ± 0.5040	0.5078	99.56 ± 0.2000	0.2009

*Each value is a mean of three observations.

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