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Development and Validation of Mefenamic Acid, Dicyclomine HCl and Pamabrom in Marketed Formulation by HPLC

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Abstract

Method has been validated by using spectrophotometric and chromatographic techniques for Mefenamic acid (MEF), Dicyclomine Hydrochloride (DCL) and Pamabrom (PABr) in bulk powder and in pharmaceutical formulations, with a high degree of specificity, selectivity and assurances, method for the drug combination has been not reported. The objectives were to develop and validate a simple, precise and accurate UV and RPHPLC method for simultaneous estimation of mefenamic acid, dicyclomine hydrochloride and pamabrom from multicomponent tablet dosage form. The first Vierdot's method was performed and absorption maxima of MEF, DCL and PABr at 285, 218 and 278 nm, respectively. Calibration graphs were established in the range of 2-24 µg/mL. The retention time were found to be 5.789, 2.522 and 4.284 min. respectively. UV and HPLC methods were developed and validated for pharmaceutical dosages forms.

Keywords: Mefenamic acid; Dicyclomine HCl; Pamabrom; HPLC; Method development

Introduction

To estimate the amount or concentration of active pharmaceutical ingredient(s) [APIs] in pure or pharmaceutical dosage form, analytical method development is a standardized laboratory procedure [1,2]. Various analytical methods that were utilized by the quality control laboratories to certify the identity, pureness, effectiveness, and performance of drug products are HPLC, UV-Spectrophotometry, HPTLC, Titration, Fluorescence spectroscopy [3]. The marketed formulation of Twagic spas Manufactured by Akums Drugs and Pharmaceuticals Ltd. and marketed by Kepler Healthcare Pvt. Ltd. This combination has been selected on the basis of publication not available in literatures.

Mefenamic acid (MEF) is chemically 2-(2,3-dimethyl phenyl amino) benzoic acid. It is utilized for the short-term treatment of mild to moderate pain from various conditions. It is also used to decrease pain and blood loss from menstrual periods. Mefenamic acid is the drug which comes under the NSAID. Mefenamic acid is non- steroidal drug anti-inflammatory drug with analgesic and antipyretic activity. It inhibits prostaglandin synthesis and competes for binding at the prostaglandin receptor site (Figure 1) [4].

Dicyclomine HCl (DCL) is chemically 2-(Diethylamino) ethyl 1,1'-bis (cyclohexyl)-1-carboxylate. It is an antispasmodic and anticholinergic (antimuscarinic) agent. Its action is achieved *via* a dual mechanism: a specific anticholinergic effect (antimuscarinic) at the acetylcholine-receptor sites, a direct effect upon smooth muscle (musculotropic) (Figure 2) [5].

Pamabrom (PABr) is chemically 2 amino-2 methyl propanol 8-bromotheophyllinate. It is a xanthine derivative and it might increase the renal blood flow by virtue of their cardiac stimulant property and vasodilator action which promotes filtration of fluid by the glomeruli. Also, produce diuresis by diminishing the tubular reabsorption of water. Interference in tubular reabsorption of Na^+ and Cl^- perhaps by acting on the enzyme concerned with the transport of these ions (Figure 3) [6].

Physical properties of the standard drugs (mefenamic acid, dicyclomine HCl, and pamabrom) are shown in Table 1.

Experimental

Chemicals and reagents

Acetonitrile and methanol were procured from the Rankem (New Delhi, India). All these chemicals and solvent were HPLC grade. These chemicals were utilized deprived of extra purification. All the required dilutions were made in the mobile phase. HPLC grade water was obtained from water purification systems ELIX 03 (MILLIPORE, USA). Unless otherwise specified, all the solutions were filtered through a 0.2 µm Ultipor N66 Nylon 6, 6 membrane filter (Pall Life Sciences, USA) prior to use.

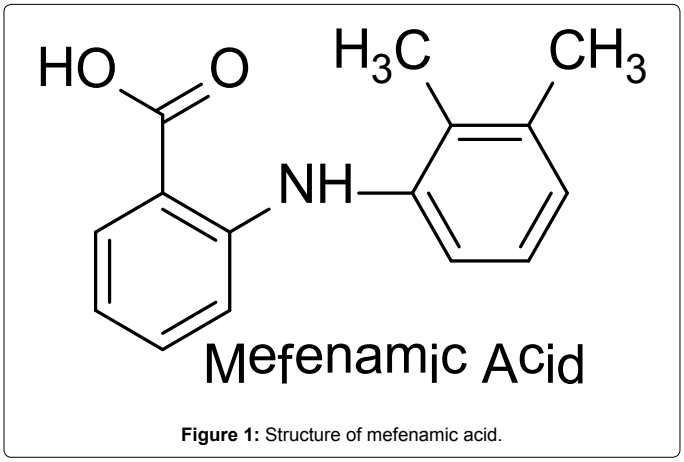

Figure 1: Structure of mefenamic acid.

Instrumentation

The analysis was accomplished on HPLC system of WATERS (Milford, USA) composed of 515 HPLC pump as a solvent delivery system equipped with Rheodyne injection valve with a 20 µL loop, WATERS 2489 UV detector set at wavelength range 190-400 nm.

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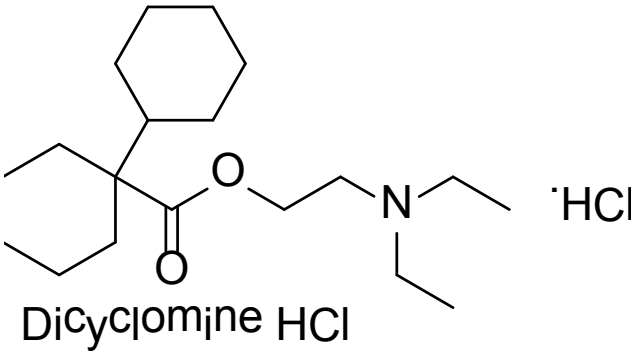

Figure 2: Structure of dicyloamine HCl.

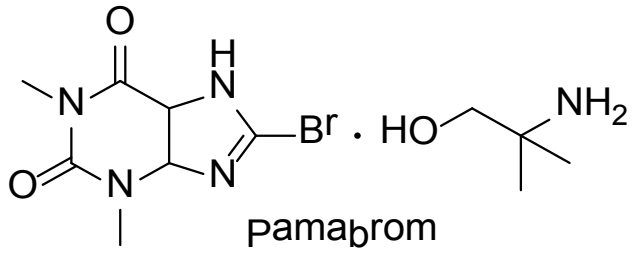

Figure 3: Structure of Pamabrom.

Chromatographic data were recorded and processed using the EMPOWER-2 software. UV-Vis double beam spectrophotometer UV-1700 Pharma Spec was utilized for entire spectrophotometric measurements, having a slit width of 1 nm, installed with UV Winlab and UV Winlab data processor and UV Probe (2.31) software. Ultra-bath sonicator (PCI analytics 3.5 L capacity) was utilized for proper mixing and sonication of stock solutions. FTIR spectrometer (Thermo Nicolet iS10, Corp. USA) with a computer loaded with EZ Omnic software, Analytical Balance (Mettler Toledo, AB204-S/FACT) and Water purification systems ELIX 03 (MILLIPORE, USA).

HPLC

Chromatographic condition: The mobile phase was prepared and filtered through Millipore filter paper (0.2 µ), apparent pH was adjusted to 5.9 using orthophosphoric acid (OPA) and acetonitrile: Potassium dihydrogen orthophosphate (20 mM) in a ratio of 70:30 v/v. Column (4.6 mm × 250 mm × 5 µm), flow rate was 1.0 mL/ min, 1300-1400 psi pressure and detector channel was set at 218 nm, injection volume was 20 µL.

Standard solution: MEF, DCL and PABr stock solutions were prepared by dissolving drug in methanol at 1 mg/mL concentration. The stock solution used to prepare desired concentration range as per sample calibration range. The final standard laboratory mixture dilution contained 10 µg/mL of MEF, 10 µg/mL of PABr and 100 µg/mL of DCL that was utilized for UV-spectroscopy scanning. The final standard laboratory mixture dilution contained 100 µg/mL.

Sample preparation: Weighed the twenty tablets and finely powdered. The equivalent weight was calculated and according to the average weight, the required drug was taken in a volumetric flask, to obtain 10 µg/mL of MEF, 10 µg/mL of PABr, 100 µg/mL of DCL in single dilution. In spiking method required amounts of MEF, DCL and PABr (API's) were added and resultant solution was filtered through Whatmann filter paper number 41. All the dilutions were prepared in methanol and absorbance were measured and analyzed. Stock solutions used to prepare desired concentration range as per sample calibration range 2-10 µg/mL of MEF, 2-10 µg/mL of PABr and 100-600 µg/mL of DCL.

Description	Mefenamic Acid	Dicyclomine HCl	Pamabrom
Appearance	White Crystalline	White Crystalline powder	White powder
Solubility	Methanol	Methanol	Methanol
pKa	4.2	8.96	0.23
Log P	5.12	5.5	5.58
Melting point	230˚C-231˚C	165˚C-166˚C	348.2˚C

Table 1: Physical properties of the standard drugs.

Results and Discussion

Vierdot's method

In this method calibration curves were obtained from all the three drugs and the absorbance of MEF, DCL and PABr were determined at 200-400 nm. The λ_{max} was found at 285, 218 and 278 nm, respectively. The linearity curves were plotted for quantitative analysis of MEF, DCL, and PABr. Linearity ranges were observed in concentration range of 2-24 µg/mL of MEF, 2-24 of PABr and 100-600 of DCL. The linear equation was The results of calibration curves were plotted and the concentration, absorbance of three drugs (MEF, DCL, and PABr) shown in and Table **2**. The overlay graph of linearity of three individual drugs and mixture is shown in Figure 4 and Figure 5. From these calibration curves, slope, intercept and correlation coefficient were obtained. Thus, regression equations were calculated. These equations were used for simultaneous estimation of MEF, DCL and PABr in standard laboratory mixture and marketed formulation. The LODs and LOQs have been calculated as per ICH guidelines. The intra-day and inter-day precision were carried out and Relative standards deviation was calculated. The method was found to be precise due to low values of the % RSD shown in Table 3.

The accuracy of the method was determined in terms of % age recovery of the standard. Recovery studies were carried out by addition of standard drug solution at the level of 80%, 100% and 120% to the pre-analyzed sample. Results of the recovery study were found to be within the acceptance criteria 100% ± 10%, indicating a good degree of sensitivity of the method towards detection of analytes in a sample. In this method, the known concentration standard drug was added to the assay sample. The amount of drug that was found calculated and the assayed amount was reduced from it, from that the recovered amount calculated. The average percentage recoveries for MEF, DCL, and PABr were obtained are shown in Table 4.

Assay determination (percentage purity w/w)

For marketed formulation, an assay was performed to check the purity of each drug in the formulation and percentage purity of the drugs was calculated. Percentage estimation for MEF, DCL and PABr was found to 96.0544%, 103.250% and 105.800%, respectively. Results are described in Table **5**. Thus, procured MEF, DCL, and PABr were found to be in pure form.

HPLC method

RP-HPLC method development by using selected mobile phase in a different ratio, in different pH and validation for the combination of MEF, DCL, and PABr. The result of regression analysis and the coefficient of determination (R^2) are given in Tables 6 and **7.** The high coefficient of determination values were 0.998, 0.999 and 0.997 for

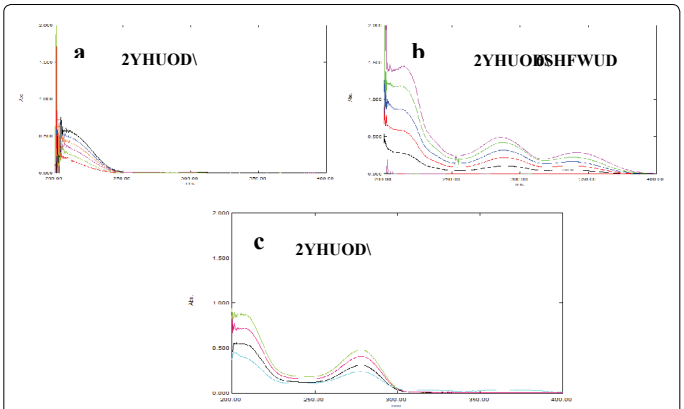

Figure 4: Overlay spectra of (a) DCL (b) MEF (c) PABr.

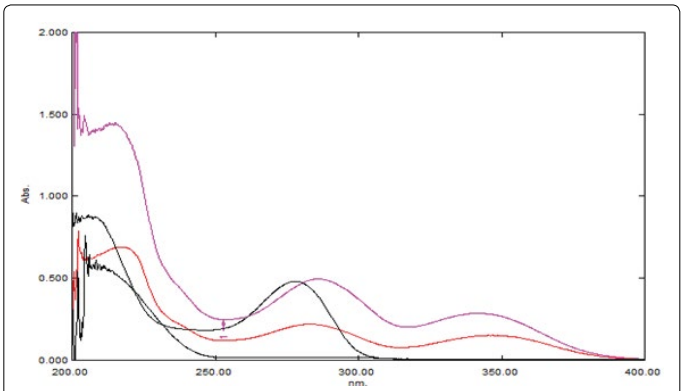

Figure 5: Overlay Spectra of MEF, DCL, PABr and Mixture.

MEF		PABr		DCL	
Concentration	Abs	Concentration	abs	Concentration	abs
2	0.135	2	0.128	100	0.131
4	0.272	4	0.216	200	0.201
6	0.401	6	0.310	300	0.280
8	0.530	8	0.400	400	0.348
10	0.647	10	0.482	500	0.412

Table 2: Concentration and absorbance of MEF, PABr and DCL.

Sr. No.	Validation Parameter	MEF	PABr	DCL
1	Absorption maxima, λ_{max} (nm)	285	278	218
2	Linearity range (µg/mL)	2-24	2-24	100-600
3	Coefficient of determination (R^2)	0.998	0.9993	0.9995
4	Regression equation (y)	y=0.037x -0.005	y=0.0007x +0.0617	y=0.0446x+0.0396
5	Slope (b)	0.037	0.0007	0.0446
6	Intercept (a)	-0.005	+0.0617	+0.0396
7	Limit of detection (µg/mL)	0.25	0.20	17.07
8	Limit of quantification (µg/mL)	0.86	0.69	56.90
9	Precision (%RSD)	Intraday=0.57 Interday=0.58	Intraday=0.54 Interday=0.55	Intraday=0.51 Interday=0.54

Table 3: Resulting parameters of Vierdot's method.

MEF, DCL and PABr, respectively (R^2>0.995) indicated good linearity between the range 0.5-15 µg/mL of MEF, 0.5-15 µg/mL of PABr and 10-200 µg/mL of DCL. The results of calibration curves were plotted and

Amount Added (µg/mL)			%Recovery study		
MEF	DCL	PABr	MEF	DCL	PABr
8	8	8	98.958	98.562	99.269
10	10	10	100.181	99.973	100.551
12	12	12	100.090	99.799	98.971
%Recovery			99.743	99.444	99.597
SD			0.681	0.769	0.839

Table 4: Recovery studies of MEF, DCL, and PABr by both methods.

S. No	Drug	Label claim (mg)	Vierdot's method	
			Amount (mg)	Estimation (%)
1	MEF	500	480.262	96.0524
2	PABr	25	26.450	105.800
3	DCL	10	10.325	103.250

Table 5: Results of the assay by using UV spectrophotometry.

the concentration, area of three drugs (MEF, DCL, and PABr) shown in Table 6.

Assay determination (percentage purity w/w)

The assay was performed to check the purity of each drug in the formulation and percentage purity of the drugs was calculated. Percentage estimation for MEF, PABr, and DCL was found to be 99.91%, 101.53%, and 99.29%, respectively and results were shown in Table 8 and Figures 6-10.

The proposed methods were validated as per International Conference of Harmonization (ICH) guidelines with respects to linearity, range, accuracy, precession, LOD (Limit of detection) and LOQ (Limit of quantification).

Specificity

Specificity was the capability of the method to accurately measure the analyte response in the existence of all potential sample components. Complete detection and separation of MEF, DCL and PABr by UV and HPLC method without any apparent shoulders, confirms the specificity of the method.

Linearity

The linearity of UV method and HPLC method was checked by preparing a standard solution of MEF, DCL, and PABr at six concentration levels in methanol using their respective stock solutions and sample preparation is also made by the same procedure. The calibration for MEF, DCL, and PABr was drawn in the concentration range of 0.5-15 µg/mL of MEF, PABr, and 10-200 µg/mL, respectively. In another hand linearity of the HPLC detector response was estimated by analyzing five concentrations of each drugs ranging between 0.5-10 µg/mL for MEF, PABr, respectively and 100-600 µg/mL of DCL. Calibration curves were found for MEF, DCL, and PABr, respectively. The linearity of both methods was validated by the under the limit value of the correlation coefficient and intercept value [7-10].

Range

The calibration ranges of MEF, DCL, and PABr were established between 0.5-15 µg/mL of MEF, PABr and 10-200 µg/mL for DCL was set for HPLC study. These ranges were obtained by consideration of the practical range, according to each drug concentration present in pharmaceutical dosages forms, to give accurate, precise and linear results [8-10].

Sr. No.	Validation Parameter	MEF	PABr	DCL
1	Absorption maxima, λ_{max} (nm)		218	
2	Linearity range (µg/mL)	80-120	20-30	8-12
3	Coefficient of determination (R^2)	0.9981	0.997	0.999
4	Regression equation (y)	y=179681x+29500	y=67409x+43599	y=613.44x-312
5	Slope (b)	179681	67409	613.44
6	Intercept (a)	+29500	+43599	-312
7	Limit of detection (µg/mL)	0.68	0.51	1.78
8	Limit of quantification (µg/mL)	2.29	1.73	5.93
9	Precision (%RSD)	0.0195	0.0146	0.0345

Table 6: Validation parameters for an RP-HPLC method for MEF, DCL, and PABr.

Amount added			%Recovery		
MEF	PABr	DCL	MEF	PABr	DCL
80	80	80	100.21	99.98	100.02
100	100	100	101.26	100.91	100.33
120	120	120	100.06	99.92	101.71
Mean recovery			100.51	100.27	100.68
SD			0.653	0.555	0.899

Table 7: Recovery studies of MEF, PABr, and DCL.

Drug Name	Label claim (mg)	Amount found (mg)	%Estimation
MEF	500	499.55	99.91
PABr	25	25.38	101.53
DCL	10	9.92	99.29

Table 8: Percentage estimation of the drugs in the marketed formulation of a ternary combination.

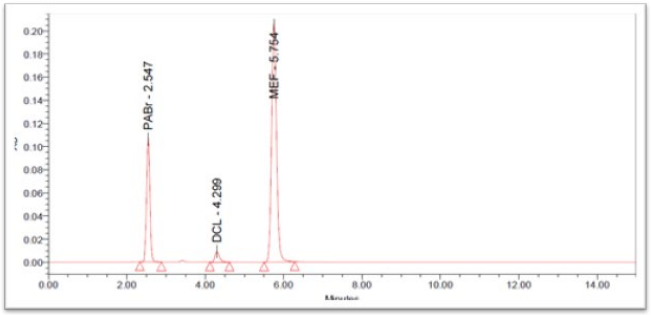

Figure 6: Chromatogram of MEF (10 µg/mL), PABr (10 µg/mL), and DCL (100 µg/mL).

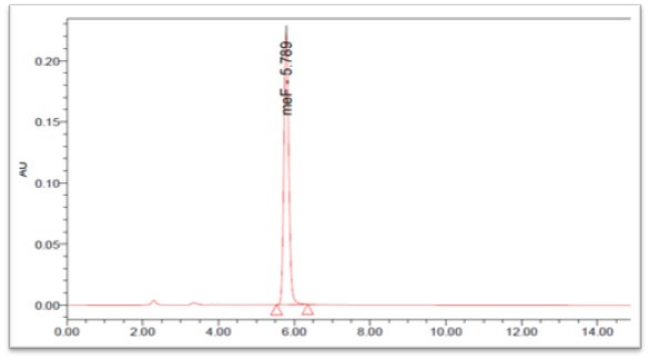

Figure 7: Chromatogram of MEF (10 µg/mL).

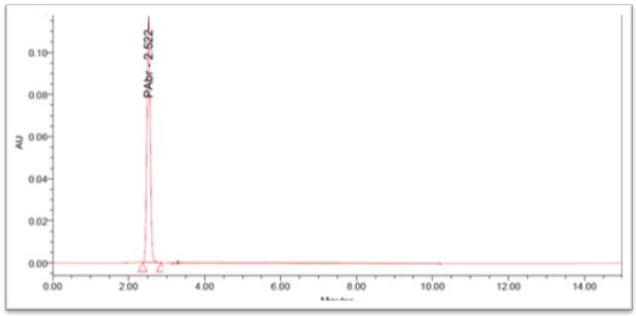

Figure 8: Chromatogram of PABr (10 µg/mL).

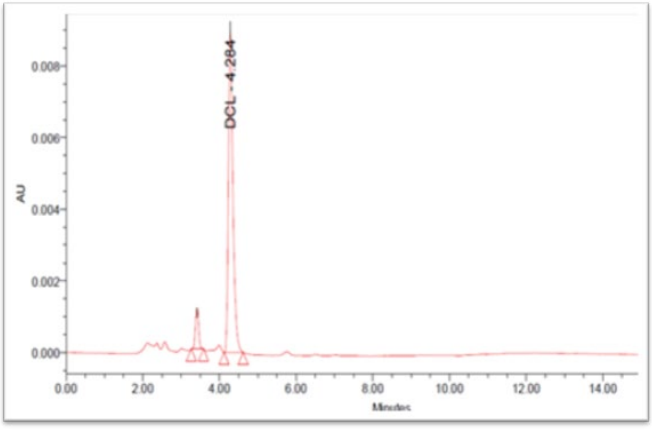

Figure 9: Chromatogram of DCL (100 µg/mL).

Detection and quantitation limits

UV Vierdot's method had higher LOD and LOQ than first-order derivative method. Where HPLC method LOD and LOQ depends on various factors like pH, Column selection, and detector sensitivity or type of detector used.

Precision

Interday and intraday precision on the concentration range of the both UV and HPLC method. The data of each concentration level were

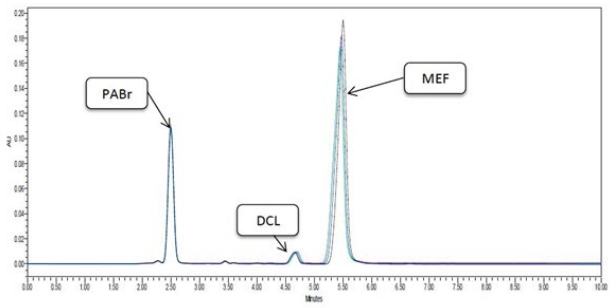

Figure 10: Overlay Chromatogram of MEF, PABr (0.5-15 µg/mL) and DCL (10-200 µg/mL).

estimated by one-way ANOVA. There was no statistically significant difference between the mean results achieved from one level of day to another at the 95% confidence level. Generally for drug substances RSD to be kept for replicate measurement is less than 1.0% and for drug product RSD to be kept for replicate measurement is less than 2.0%.

As per ICH guidelines, minimum nine determination to be access for repeatability, which should cover the specified range. Either three replicate for each three concentration level or six determinations over the concentration at specification level at 100% [8-10].

Accuracy

Accuracy was performed by using spiking method (standard addition method). It was carried out 80%, 100% and 120% for all three drugs in UV and HPLC method. The mixtures were analyzed and results obtained were compared with standard solution results and suggested the good accuracy of the proposed methods.

As per ICH guideline, accuracy should cover minimum nine determinations with at least three concentration level i.e. for each three concentration, three replicate determinations are mandatory [8-10].

Robustness

Spectrophotometric methods were carried out at room temperature by using AR grade methanol that consistently produces a result without any alteration [10].

Conclusion

UV spectrophotometer and HPLC methods were developed and validated for the simultaneous determination of MEF, DCL, and PABr in pharmaceutical dosages forms. The method was found to be simple, precise and rapid. In UV spectrophotometer, Vierdot's method and HPLC method may offer an advantage for selective determination of MEF, DCL, and PABr in the presences of pharmaceutical dosages forms or any variety of matrices. A comparative study of the use of HPLC and UV spectrophotometer methods for the resolution of a ternary mixture of MEF, DCL, and PABr has been accomplished. The results of Vierdot's method as well as HPLC method have a significant resolution. The reported method is quite economic as the range of retention time for all the three drugs is 2.547-5.754 min which reduced time of method and excessive use of solvent.

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