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Der Pharmacia Lettre, 2010, 2(6): 28-36
(http://scholarsresearchlibrary.com/archive.html)

ISSN 0975-5071
USA CODEN: DPLEB4

Validated HPTLC Method for simultaneous quantitation of Paracetamol, Tramadol and Aceclofenac in tablet formulation

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ABSTRACT

This paper describes a new, simple, precise, and accurate HPTLC method for simultaneous quantitation of Paracetamol (PARA), Tramadol (TRA) and Aceclofenac (ACF) as the bulk drug and in tablet dosage form. Chromatographic separation of the drugs was performed on aluminum plates precoated with silica gel 60 F_{254} as the stationary phase and the solvent system consisted of toluene: ethyl acetate: formic acid 7: 2: 0.5 (v/v/v). Densitometric evaluation of the separated zones was performed at 261 nm. The two drugs were satisfactorily resolved with R_F values 0.19 ± 0.02, 0.40 ±0.02 and 0.53 ± 0.02 for PARA, TRA and ACF, respectively. The accuracy and reliability of the method was assessed by evaluation of linearity (487.5–2437.5 ng.spot^{-1} for PARA, 56.25-281.25ng.spot^{-1} and 150-750 ng.spot^{-1} for ACF), precision (intra-day RSD 0.39-1.95 % and inter-day RSD 0.56–1.88 % for PARA, and intra-day RSD 1.02-1.92 % and inter-day RSD 1.11–1.91 % for TRA and intra-day RSD 1.80-1.99 and inter day RSD 0.90-1.97 % for ACF), in accordance with ICH guidelines.

Keywords: Thin layer Chromatography, Densitometry, Validation and Quantification, Paracetamol, Tramadol and Aceclofenac.

INTRODUCTION

TRA (Fig. 1a) is chemically 1*R*,2*R*)-2-[(dimethylamino)methyl]- 1-(3-methoxy phenyl) cyclohexanol. It is believed that it works through modulation of the noradrenergic and serotonergic systems in addition to its mild agonism of the μ-opioid receptor. ACF (Fig. 1b) is chemically 2-[2-[2-[(2,6- dichlorophenyl)amino]phenyl]oxyacetic acid. It is used for the relief of pain and inflammation in rheumatoid arthritis, osteoarthritis and ankylosing spondylitis [3]. PARA(Fig. 1c) is chemically N-(4-hydroxyphenyl)acetamide. Acetaminophen is thought to act

primarily in the CNS, increasing the pain threshold by inhibiting both isoforms of cyclooxygenase, COX-1 and COX-2, enzymes involved in prostaglandin (PG) synthesis. Unlike NSAIDs, acetaminophen does not inhibit cyclooxygenase in peripheral tissues and, thus, has no peripheral anti-inflammatory affects.

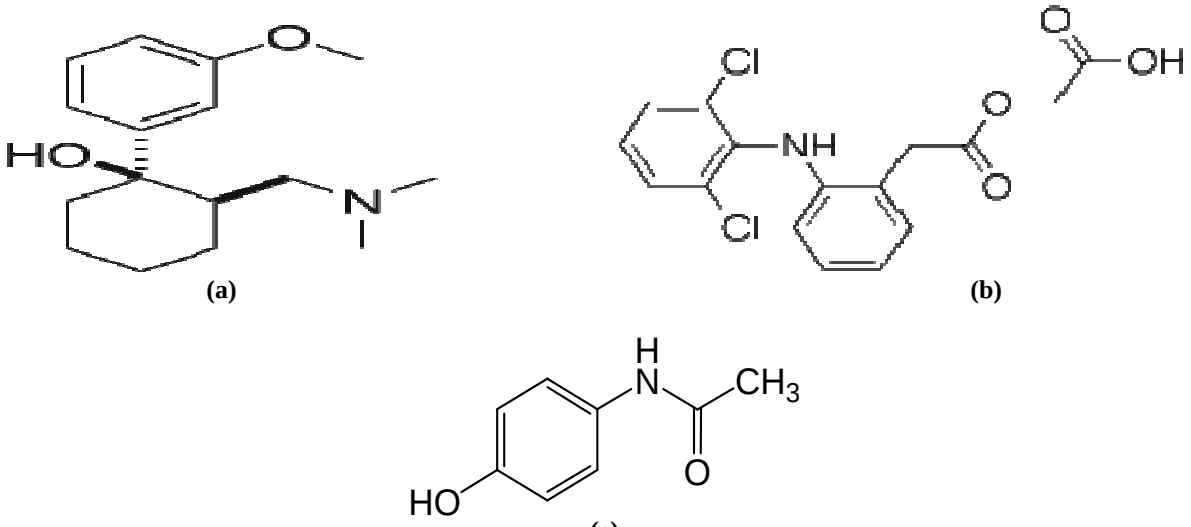
Literature review reveals that methods have been reported for analysis of PARA by spectrophotometry [18], chemiluminecsence [19] and for ACF methods reported are spectrophotometry [20], spectrofluorimetry [20], capillary electrophoresis in plasma [21], dissolution [11], HPLC [14] and HPTLC [16] either alone or in combination with other drugs. To date, there have been no published reports about the simultaneous quantitation of PARA, TRA and ACF by HPTLC in bulk drug and in pharmaceutical dosage forms. This present study reports for the first time simultaneous quantitation of PARA, TRA and ACF by HPTLC in bulk drug and in pharmaceutical dosage forms. The proposed method is validated as per ICH guidelines.

MATERIALS AND METHODS

Working standards of pharmaceutical grade TRA (batch no. 0410708), PARA(bach no.260738) and ACF (batch no. 18060131) were obtained as generous gifts from Glenmark Pharmaceuticals Limited, Kurkumbh (India) and Suyash Labs, Tarapure, (Thane, Maharastra, India) respectively. It was used without further purification and certified to contain 98 – 99%, 97-99% and 99 - 101 % (w/w) on dry weight basis for PARA,TRA and ACF, respectively. Fixed dose combination tablets (HIFENAC) containing 325 mg PARA , 37.5 mg TRA and 100mg of ACF were procured from INTAS Pharmacceuticals Ltd., India. All chemicals and reagents of analytical grade were purchased from Merck Chemicals, Mumbai, India.

The samples were spotted in the form of bands of width 6 mm with a Camag 100 microlitre sample (Hamilton, Bonaduz, Switzerland) syringe on silica gel precoated aluminum plate 60 F_{254} plates, [10 cm × 10 cm with 250 µm thickness; E. Merck, Darmstadt, Germany)] using a Camag Linomat V (Switzerland) sample applicator. The plates were prewashed with methanol and activated at 110^{o} C for 5 min prior to chromatography. A constant application rate of 0.1 $\mu L.s^{-1}$ was used and the space between two bands was 6 mm. The slit dimension was kept at 5 mm × 0.45 mm and the scanning speed was 10 $mm.s^{-1}$. The monochromator bandwidth was set at 20 nm, each track was scanned three times and baseline correction was used. The mobile phase consisted of toluene: ethyl acetate: formic acid (7: 2.: 0.5, v/v/v) and 11.5 mL of mobile phase was used per chromatography run. Linear ascending development was carried out in a 20 cm × 10 cm twin trough glass chamber (Camag, Muttenz, Switzerland) saturated with the mobile phase. The optimized chamber saturation time for the mobile phase was 30 min at room temperature (25^{o} C ± 2) at relative humidity of 60 % ± 5. The length of each chromatogram run was 8 cm. Following the development, the TLC plates were dried in a current of air with the help of an air dryer in a wooden chamber with adequate ventilation. The flow rate in laboratory was maintained unidirectional (laminar flow, towards the exhaust). Densitometric scanning was performed using a Camag TLC scanner III in the reflectance-absorbance mode at 260 nm (Fig. 2) and operated by CATS software (V 3.15, Camag). The source of radiation used was deuterium lamp emitting a continuous UV spectrum between 190 and 400 nm. Concentrations of the

compound chromatographed were determined from the intensity of the diffused light. Evaluation was by peak areas with linear regression.



(a) **(b)**

(c)

Fig. 1 Structure of (a) TRA (b) ACF (c) PARA

2.3. Preparation of Standard Stock Solutions

Standard stock solutions of concentration 32.5 mg/mL of PARA, 3.75 mg/mL of TRA and 10 mg/mL of ACF were prepared separately using methanol. The stock solution was stored at 2-8 °C protected from light.

2.4. Optimization of the HPTLC method

The TLC procedure was optimized with a view to develop a simultaneous assay method for TRA, ACF and PARA. The mixed standard stock solution (3.25 mg/mL of PAR, 0.375 mg/mL of TRA and 1.0 mg/mL of ACF) was spotted on to TLC plates and run in different solvent systems. To develop a simultaneous assay method for TRA, ACF and PARA was very critical because of different polarities of these drugs. Finally, the mobile phase consisting of toluene: Ethyl acetate: formic acid in the ratio of 7: 2: 0.5, v/v/v was found optimum. Densitometric scanning was done at 254 nm as all drugs showed maximum response at that wavelength *(Fig.4.1)*. In order to reduce the neckless effect HPTLC chamber was saturated for 20 min using saturation pads. The mobile phase was run up to a distance of 8 cm; which takes approximately 20 min for complete development of the HPTLC plate.

2.5. Validation of the method

Validation of the optimized HPTLC method was carried out with respect to the following parameters.

2.5.1. Linearity and range

From the working standard stock solution (3.25 mg/mL of PARA, 0.375 mg/mL of TRA and 1 mg/mL of ACF), 1 to 5 µl solutions were spotted on HPTLC plate to obtain final concentration of 487.5- 2437.5 ng/spot, 56.25-281.25 ng/spot and 150-750 ng/spot for PARA, TRA and ACF respectively. Each concentration was applied in triplicate on the HPTLC plate. . The plate was

then developed using the previously described mobile phase and the peak areas were plotted against the corresponding concentrations to obtain the calibration curves.

2.5.2. Precision

The precision of the method was verified by repeatability and intermediate precision studies. Repeatability studies were performed by analysis of three different concentrations by HPTLC (487.5, 1462.5, 2437.5 ng/spot for PARA, 56.25, 168.75, 281.25 ng/spot for TRA and 150, 450 and 750 ng/spot for ACF) of the drug five times on the same day. The intermediate precision of the method was checked by repeating studies on three different days.

2.5.4. Robustness of the method

Following the introduction of small changes in the mobile phase composition (± 0.1 mL for each component), the effects on the results were examined. Mobile phases having different compositions, e.g. toluene: Ethyl acetate: formic acid (7.1: 1.9:0.4, v/v/v), (6.9: 2: 0.6, v/v/v), (7: 1.9: 0.4, v/v/v), (7: 2: 0.5, v/v/v), were tried and chromatograms were run. The amount of mobile phase was varied over the range of ± 5 %. The plates were prewashed with methanol and activated at 110°C for 2, 5, and 7 min respectively prior to chromatography. The time from spotting to chromatography and from chromatography to scanning was varied by ± 10 min. The robustness of the method was determined at three different concentration levels 487.5, 1462.5, 2437.5 ng/spot for PARA, 56.25, 168.75, 281.25 ng/spot for TRA and 150, 450 and 750 ng/spot for ACF.

2.5.5. Specificity

Analysis of standard drug and test samples were done. The spot for PARA, TRA and ACL in the samples was confirmed by comparing the R_f and spectrum of the spot with that of a standard. The peak purity of PARA, TRA and ACL was determined by comparing the spectrum at three different regions of the spot i.e. peak start (S), peak apex (M) and peak end (E).

2.5.6. Recovery

Recovery studies of the drugs were carried out for the accuracy parameter. These studies were carried out at three levels i.e. multiple level recovery studies. Apply the method to drug sample (PARA, TRA and ACL combination tablet) to which know amount of PARA, TRA and ACL standard powder corresponding to 50%, 100% and 150% of label claim had been added (Standard addition method), mixed & analyzed by running chromatogram in optimized mobile phase.

2.6. Analysis of a marketed formulation

To determine the content of PARA, TRA and ACL in conventional tablet (Brand name: HIFENAC TA, Label claim: 325 mg of paracetamol, 37.5 mg of Tramadol and 100 mg of Aceclofenac per tablet), twenty tablets were weighed, their mean weight determined and finely powdered. The weight of the tablet triturate equivalent to 325 mg of PARA, 37.5 mg of TRA and 100 mg of ACL was transferred into a 50 mL volumetric flask containing 20 mL methanol, sonicated for 30 min and diluted to 50 mL with methanol. The resulting solution was centrifuged at 3000 rpm for 5 min and the drug content of the supernatant was determined (13, 1.5 and 4.0 mg/mL for PARA, TRA and ACL, respectively). Then 5 mL of the above filtered -solution was diluted to produce a concentration of 1300, 150 and 400 µg/mL for PARA, TRA and ACL,

respectively and 1 µL of this solution (1300, 150 and 400 ng/spot for PARA, TRA and ACL, respectively) was applied to a HPTLC plate which was developed in optimized mobile phase. The analysis was repeated in triplicate. The possibility of excipient interference with the analysis was examined.

RESULTS AND DISCUSSION

3.1 Validation

The results of validation studies on simultaneous estimation method developed for PARA, TRA and ACF in the current study involving toluene: ethyl acetate: formic acid (7: 2: 0.5, v/v/v) as the mobile phase for HPTLC are given below.

3.1.1. Linearity

The drug response was linear by HPTLC (Table 1) over the concentration range between 487.5-2437.5 ng/spot, 1906-6546 ng/spot and 150-750 ng/spot for PARA, TRA and ACL, respectively by HPTLC (r^2 = 0.997, 0.995 & 0.999 for PARA, TRA and ACL, respectively) .

3.1.2. Precision

The results of the repeatability and intermediate precision experiments are shown in Table 2. The developed method was found to be precise as the RSD values for repeatability and intermediate precision studies were < 2 %, respectively as recommended by ICH guidelines.

3.1.3. Robustness of the method

The standard deviation of the peak areas was calculated for each parameter and the % RSD was found to be less than 2 %. The low values of the % RSD, as shown in Table 3 indicated robustness of the method by HPTLC.

3.1.4. Specificity

The peak purity of PARA, TRA and ACL was assessed by comparing their respective spectra at the peak start, apex and peak end positions of the spot i.e., r (S, M) = 0.9998 and r (M, E) = 0.9997 and r (S, M) = 0.9999 and r (M, E) = 0.9997, respectively. A good correlation (r = 0.9997) was also obtained between the standard and sample spectra of PARA, TRA and ACL respectively.

3.1.6. Recovery Studies

Good recoveries of the PARA, TRA and ACL were found in the range from 98.12 to 99.23 % by HPTLC were obtained at various added concentrations. The average recovery of three levels (nine determinations) PARA, TRA and ACL were 98.39 % , 98.58 % & 98.61 % , respectively by HPTLC (Table 4)

3.2. Analysis of marketed formulation

Experimental results of the amount of PARA, TRA and ACL in tablets, expressed as a percentage of label claims were in good agreement with the label claims thereby suggesting that there is no interference from any of the excipients which are normally present in tablets. The drug content for PARA, TRA and ACL were found to be 98.75 %, 98.4 % & 99.25 %, respectively by HPTLC. Two different lots of PARA, TRA and ACL combination tablets were analyzed using the proposed procedures.

Table 1. Precision study for PARA, TRA & ACL

Drug	Actual conc. (µg/mL)	Intra-day precision (n=6)			Inter-day precision (n=6)		
		Observed conc. (µg/mL)	S.D.	RSD (%)	Observed conc. (µg/mL)	S.D.	RSD (%)
PARA	487.5	501.44	92.587	1.95	489.64	85.02	1.88
	1462.5	1474.2	127.95	1.37	1463.08	51.82	0.56
	2437.5	2342.43	47.32	0.39	2347.55	76.32	0.64
TRA	56.25	55.98	37.564	1.92	54.88	32.62	1.91
	168.75	171.34	45.03	1.02	168.96	66.74	1.75
	281.25	268.65	85.59	1.43	269.63	58.19	1.11
ACL	150	155.7	30.006	1.80	153.9	26.002	1.81
	450	470.34	68.02	1.81	462.91	65.808	1.97
	750	728.25	108.864	1.99	721.8	40.9304	0.90

Table 2. Robustness Testing of PARA, TRA & ACL (n=3)

Parameter	SD of peak area			% RSD		
	PARA	TRA	ACL	PARA	TRA	ACL
Mobile phase composition (± 0.1 ml)	7.22	3.25	3.84	0.46	0.67	1.07
Amount of mobile phase (± 5%)	2.13	4.37	4.08	0.31	1.12	0.863
Time from spotting to chromatography (± 10 min)	1.26	2.69	5.14	0.631	2.33	1.42
Time from chromatography to scanning (± 10 min)	3.28	1.66	2.72	1.17	1.02	1.17

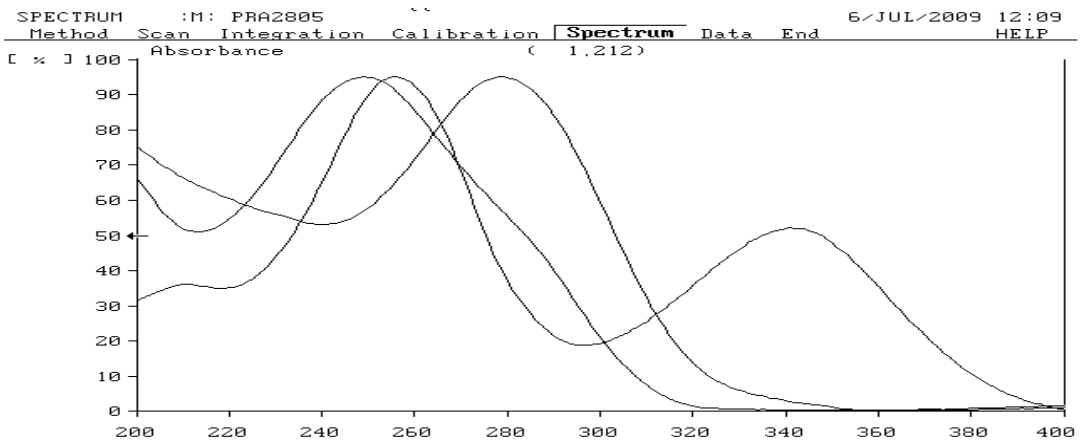
Table 3. Recovery studies PARA, TRA and ACL: (n=3)

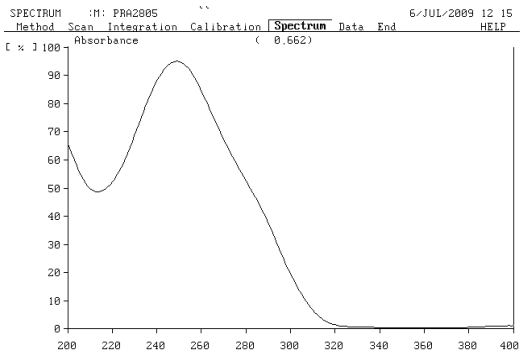
Drug (mg/Tab)	Amount added in mg (%)	Total amount (mg)	Amount recovered (mg)	S.D.	RSD (%)	Recovery (%)
PARA	162.5(50)	487.5	480.96	2.58	0.54	98.66
(325 mg)	325(100)	650	574.275	2.79	0.48	98.40
	487.5(150)	812.5	721.31	2.82	0.39	98.12
TRE	18.75(50)	56.25	54.70	0.14	0.62	98.37
(37.5 mg)	37.5(100)	75	53.61	0.26	0.91	99.23
	56.25(150)	93.75	54.01	0.16	0.45	98.14
ACL	50(50)	150	131.95	0.53	0.40	98.50
(100 mg)	100(100)	200	156.06	0.80	0.51	99.10
	150(150)	250	206.95	0.81	0.39	98.23

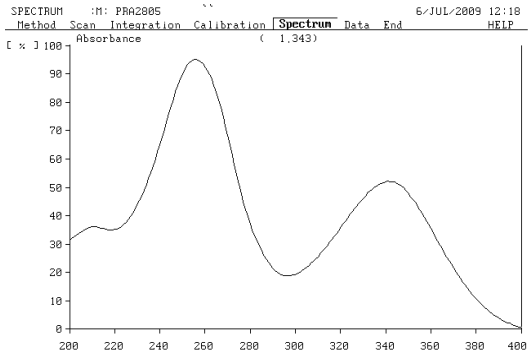
Table 4. Analysis of commercial formulation

DRUG	LABEL CLAIM	DRUG CONTENT % ± S.D.	%RSD
Paracetamol	325mg	98.75	0.45
Tramadol	37.5mg	98.40	0.88
Aceclofenac	100mg	99.25	1.18

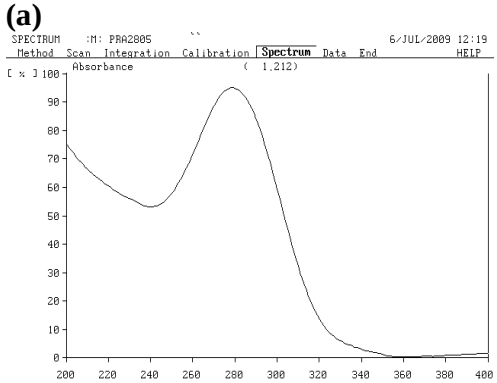
[a] *(n=3)*







(a) **(b)**



(c)

Overlain in situ spectra of PARA, TRA and ACL
λmax of PARA: 249 nm, λmax of TRA: 256nm and λmax of ACL: 278nm
Final wavelength was found to be 261 nm.

Fig. 2 Representative spectrum of (a) TRA (b) ACF (c)PARA (d) In-situ overlain spectrum of TRA, ACF and PARA

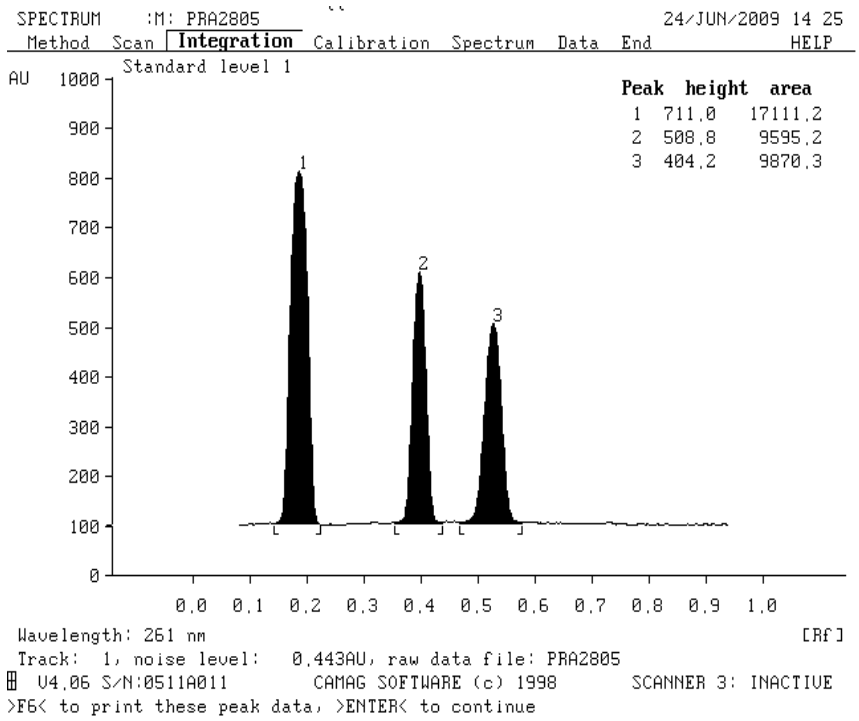


Fig. 3 Densitogram of PARA (R_F 0.46), TRA(0.19)and ACF (R_F 0.58) of formulation (HIFENAC TA) showing no interference of excipients in analysis.

CONCLUSION

The developed HPTLC method is simple, precise, specific and accurate for quantitative determination of Paracetamol, Tramadol and Aciclofenac as bulk drug and in pharmaceutical formulation without any interference from the excipients. The chromatographic method is validated according to ICH guidelines. Statistical tests indicates that the proposed method reduces the duration of analysis and appears to be equally suitable for the routine analysis in pharmaceutical formulation in quality control laboratories, where time factor is important.

Acknowledgement
The authors would like to thank, Glenmark Pharma Ltd. (Kurkumbh, India) and Suyash Labs, Tarapur (Thane, Maharastra, India), for providing a gift sample of standard Paracetamol, Tramadol and aceclofenac. The authors would like to thank, Dr. K. R. Mahadik, Principal, Poona College of Pharmacy, Pune, India for providing necessary facilities to carry out the work. Prafulla P. Apshingekar is very thankful to AICTE for financial assistance during M.Pharm.

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