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Spectroscopic Method for the Determination of Drugs Containing Phenol Group by Using 2, 4- Dinitro Phenylhydrazine

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ABSTRACT

A spectroscopic method has been proposed for the determination of two phenolic drugs; Phenylephrine hydrochloride and Pyridoxine hydrochloride. The method is based on the oxidation of 2, 4- Dinitro phenylhydrazine and coupling of the oxidized product with drugs to give intensely colored chromogen. Under the proposed optical condition, Beer's law was obeyed in the concentration range of 2.5 - 30 µg $mL^{-1}$and 5 – 20 µg mL^{-1} for Phenylephrine Hydrochloride and Pyridoxine Hydrochloride respectively. The limit of detection (LOD) and limit of quantification (LOQ) were 0.3, 1.95 µg mL^{-1} and 0.95, 0.64 µg mL^{-1} in the same order. No interference was observed from common pharmaceutical adjuvants. The suggested method was further applied for the determination of drugs in commercial pharmaceutical dosage forms, which was compared statistically with reference methods by means of t- test and F- test and were found not to differ significantly at 95% confidence level. The procedure is characterized by its simplicity with accuracy and precision.

Keywords: 2, 4- Dinitro phenylhydrazine, Phenylephrine hydrochloride, Pyridoxine hydrochloride and Spectrophotometry.

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INTRODUCTION

Phenylephrine hydrochloride (PEH) (Nec-synephrine) is (R)-1-(3-hydroxyphenyl)-2-methyl-aminoethanol hydrochloride [61-76-7]. It is closely related chemically to epinephrine. It is a useful vasoconstrictor of sustained action with little effect on the myocardium or the central nervous system. It is used by topical application in nose drops. Subcutaneous injection has been employed extensively to prevent hypotension during spinal anaesthesia and for the treatment of orthostatic hypotension. Many procedures are known for the qualitative detection and for the quantitative determination of Phenylephrine hydrochloride. Among the several analytical methods reported are anodic stripping voltametric[1], voltametric[2], Spectrophotometry[3,4,5,6], Reversed phase HPLC[8,9,10],Sequential injection spectrophotometry[11] and High performance liquid chromatographic methods[12, 13]. Pyridoxinehydrochloride (POC), (5-hydroxy-6-methylpyridine-3, 4-dimethyl) dimethanol hydrochloride is used in the treatment of sideroblastic anemia's; it is readily absorbed from the gastrointestinal tract following oral administration and is converted to the active forms, pyridoxal phosphate and pyridoxamine phosphate, which are stored mainly in the liver where there is oxidation to 4-pyridoxic acid and other metabolites that are excreted in the urine. It is involved in amino acid as well as carbohydrate and fat metabolism. It is used in a variety of disorders, including the treatment of depression. The techniques used in this connection include capillary electrophoresis[14], Zero-crossing[15], chemiluminiscent[16], Derivative spectrophotometric and Differential derivative spectrophotometric[17,18], High performance liquid chromatography[19,20], voltametric[21], Spectrofluorimetric and spectrophotometric[22,23] and spectrophotometry[24 – 26]. P. Nagaraja et.al used the same reagent 2, 4- Dinitro phenyl hydrazine for the spectrophotometric determination of drugs containing phenol group such as salbutamol sulphate, Ritrodine hydrochloride, Amoxicillin trihydrate, Isoxsuprine hydrochloride in the year 2010[27]. The present paper reports a rapid and sensitive method for the colorimetric estimation by 2, 4- Dinitro phenyl hydrazine (2, 4- DNP). 2, 4- Dinitro phenyl hydrazine also known as Brady's reagent, has been used for the characterization of aldehydes and ketones by hydrazone formation. We report here its application in the estimation of phenolic drugs. The method is based on the oxidation of 2, 4- DNP to produce diazonium cation which couples with phenolic drugs to yield highly absorbing chromogen. Studies on different variables affecting the reaction were optimized. The developed method is simple, accurate and applicable for their determination in pharmaceutical formulations. The structure of studied drugs is as shown in (Figure 1 and 2).

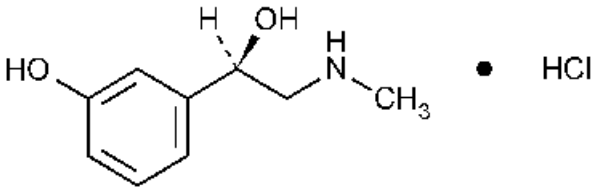


Figure 1: Phenylephrine hydrochloride

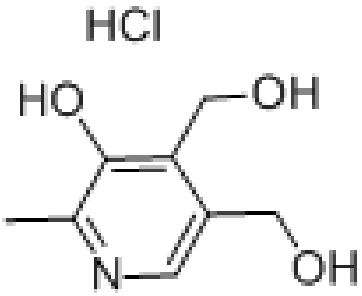


Figure 2: Pyridoxine hydrochloride

MATERIALS AND METHOD

A BL 198 Bio spectrophotometer (UV – VIS) with 1.0 cm matched quartz cuvettes cells was used for all absorbance measurements.

Reagents and solutions

Analytical reagent grade chemicals and double distilled water were used throughout the experiment. Phenylephrine hydrochloride (Amrut Drug Research lab Pvt. Ltd, Surat) and Pyridoxine hydrochloride (Mylon, Brazil) were purchased and used as received. Stock solutions of each drug containing 100 µg mL^{-1} were prepared by dissolving 10mg of the respective drugs in 100 mL of water. The solutions were further quantitatively according to their linearity range. The pharmaceutical preparations were purchased from a local market and analyzed.

Preparation of 2, 4-DNP reagent:

A 0.04% w/v of the reagent solution was freshly prepared by dissolving 0.04g of 2, 4-DNP (SRL chemicals, India) in 4 mL of conc.H_2SO_4 respectively and diluting to 100 mL with water.

Preparation of oxidizing agent:

A 4% w/v potassium iodate (Loba-chemicals, India) solution was prepared by dissolving suitable quantity in water.

Preparation of 10M NaOH:

A 100 mL 10M NaOH was prepared by dissolving 40g in 100 mL of water.

Procedure for pharmaceutical formulations

For the analysis of an injection, the requisite amount was transferred to a 100 mL volumetric flask

and diluted with distilled water. The drug content in the diluted solution was determined as described under the general procedure.

For the analysis of a tablet, twenty tablets was weighed, powdered and mixed thoroughly. A quantity equivalent to 10 mg of each drug was transferred to 100 mL volumetric flask, dissolved in water, shaken well, sonicated and made up to the volume with water. The resultant solution was filtered and analyzed as described under general procedure.

General procedure

Accurately measured quantity of each drug containing 100 µg mL^{-1} were prepared by dissolving 10 mg of the respective drugs in 100 mL of water. The solutions were further diluted quantitatively according to their linearity range. The suitable volume of Phenylephrine hydrochloride and Pyridoxine hydrochloride were transferred from stock solution to 10 mL volumetric flasks, which could be diluted quantitatively to obtain 2.5 - 30 µg mL^{-1} and 5 – 20 µg mL^{-1} respectively. To each flask containing drugs in the order mentioned above 1.0 and 2.0 mL of 2, 4 DNP (0.04%) and 1.0 and 1.0 mL of KIO_3 (4%) were added, Which were made alkaline by adding 1.0 mL each of NaOH (10M). The red color hence developed was further diluted to the volume with water.

RESULTS AND DISCUSSION

Spectral Characteristic

The absorption spectra of the reaction product of oxidized 2,4 DNP with drugs show maximum absorption (λ_{max}) at 530 and 520 nm for Phenylephrine hydrochloride and Pyridoxine hydrochloride respectively. The blank solution was slightly yellow in color that had negligible absorbance at the λ_{max} in which the drugs were analyzed. The thus formed color was stable for 30 min. A temperature range of 20 – 30^0 C is preferred for the reaction. The absorption spectra for Phenylephrine hydrochloride and Pyridoxine hydrochloride is as shown in the (Figure 3).

Reaction sequence and Stoichiometric relationship

The 2, 4 DNP is oxidized by KIO_3 to give diazonium cation that reacts with drugs by electrophilic substitution at the phenolic ring to give deep colored chromogens. The proposed reaction sequence for Phenylephrine hydrochloride is as shown in the scheme 1.

Pyridoxine hydrochloride also reacts in the same manner like Phenylephrine hydrochloride

Optimum reaction condition

By varying one and keeping other experimental parameters and the amount of drug constant, the effect of 2, 4 DNP, oxidizing agent and sodium hydroxide were studied. Maximum color intensity was obtained when 0.8 – 1.0 mL, 1.8 – 2.0 mL of 2, 4 DNP and 0.9 – 1.1 mL, 0.8 – 1.0 mL of

KIO_3 were added to Phenylephrine hydrochloride and Pyridoxine hydrochloride respectively. Different concentrations of NaOH was used for maximum color development and was found that 1 mL of 10 M NaOH was optimum for both Phenylephrine hydrochloride and Pyridoxine hydrochloride respectively. During the analysis the quantities of the reagents as mentioned in the general procedure were taken. The effect of 2. 4 DNP, KIO_3 and NaOH on the studied drugs is as shown in the (Figure 4, 5, 6)**.**

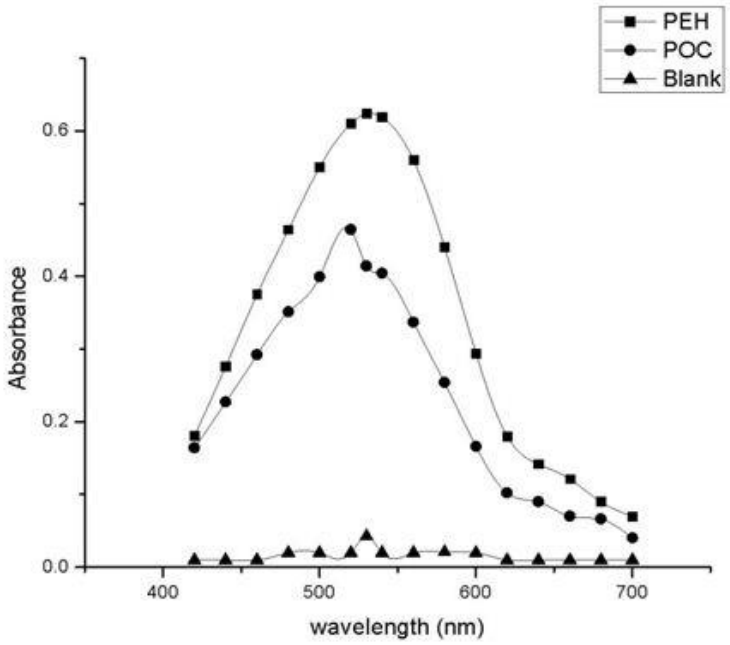
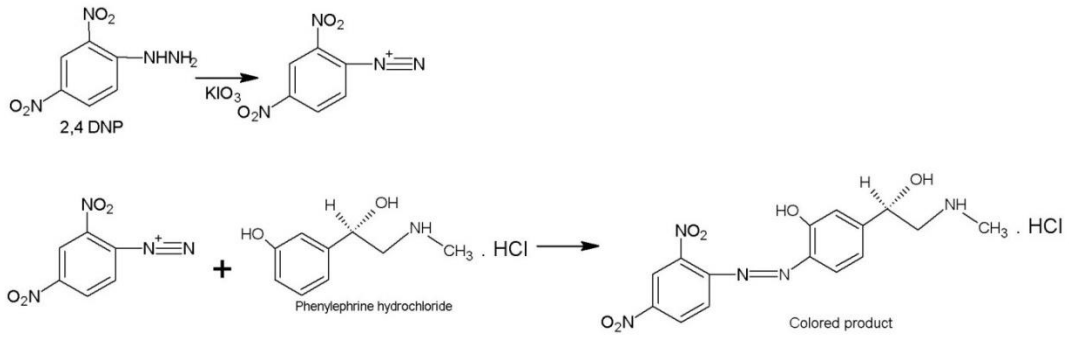


Figure 3: Absorption spectra for PEH (20 µg/mL) and POC (15 µg/mL) and blank against distilled water.



Scheme 1: The proposed reaction sequence for Phenylephrine hydrochloride

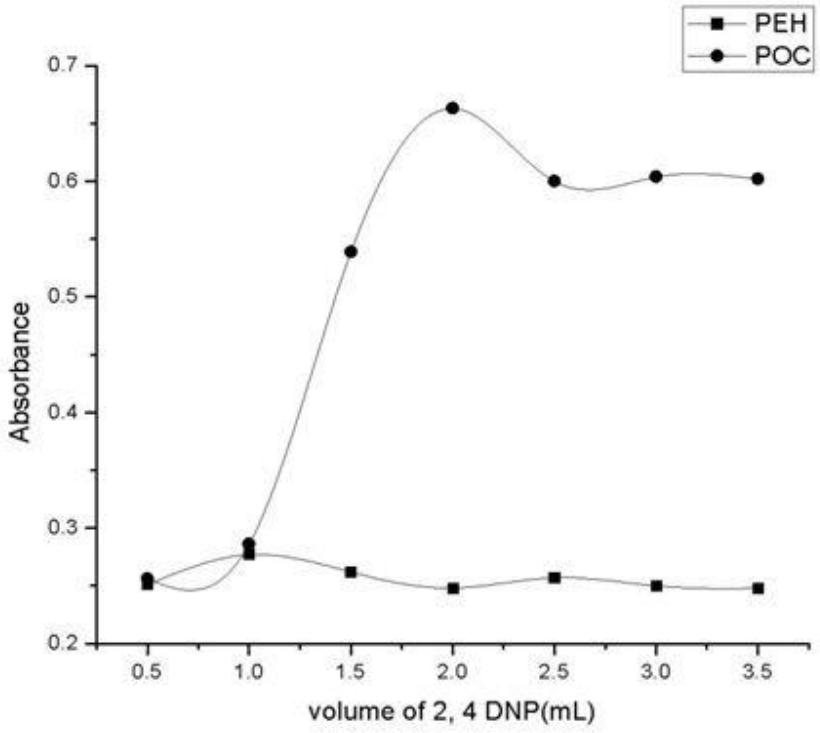


Figure 4: Effect of volume of 2,4 DNP on the reaction product with PEH (10 µg/mL) and POC (17.5 µg/mL)

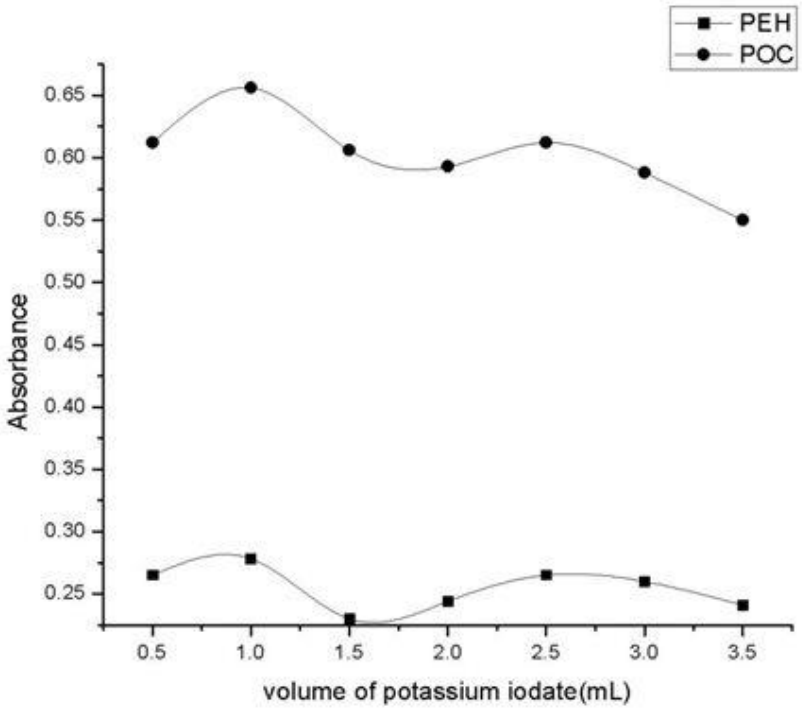


Figure 5: Effect of volume of KIO_3 on the reaction product with PEH (10 µg/mL) and POC (17.5 µg/mL)

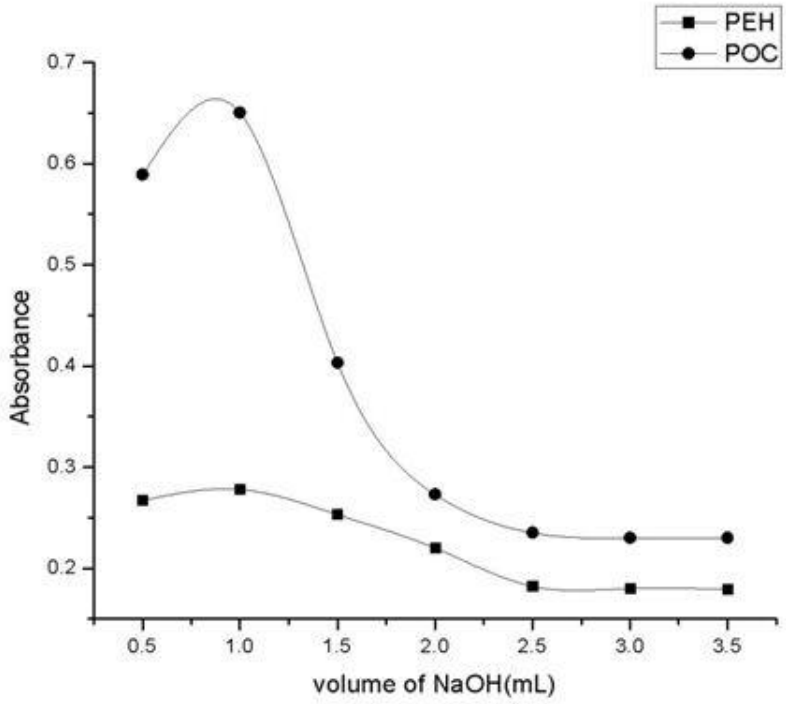


Figure 6: Effect of volume of NaOH on the reaction product with PEH (10 µg/mL) and POC (17.5 µg/mL)

Validation of the proposed method

Linearity, Detection and Quantification limit

Calibration graphs were constructed using standard solutions under optimum condition. A linear relationship was observed between the absorbance and concentration of drugs from 2.5 - 30 µg mL^{-1} and 5 – 20 µg mL^{-1} Phenylephrine hydrochloride and Pyridoxine hydrochloride respectively.

Sensitivity

Sensitivity parameters such as apparent molar absorptivity, sandell's sensitivity values and the limit of detection and quantification are calculated as per the current ICH guidelines[28]. The optical characteristics and parameters are given in Table 1, that speaks of the excellent sensitivity of the proposed method. The limit of detection (LOD) and limit of quantification(LOQ) were calculated according to the guidelines using the formulae

$$LOD = 3.3\sigma/ S, \qquad LOQ = 10\sigma/ S$$

Where σ is the standard deviation of reagent blank determination, and S is the slope of the calibration curve.

Interference studies

The effect of common excipients used in the pharmaceutical preparation were studied by analyzing synthetic sample solutions containing the quantity of drugs as mentioned in Table 2 in presence of

100 fold more concentration of each excipients. The tolerance limit was defined as the concentration which gave an error of ± 3.0% in the determination of drugs. The common excipients such as starch, dextrose, lactose, talc, magnesium stearate, had no effect in the analysis.

Table 1: Optical characteristics and parameters of the studied drugs.

Parameters	Optical characteristics	
	Phenylephrine hydrochloride(PEH)	**Pyridoxine hydrochloride(POC)**
Color	red	red
$\lambda_{max\ (nm)}$	530	520
Beer's law limit (µg mL^{-1})	2.5 - 30	5-20
Molar absorptivity (L mol^{-1} cm^{-2})	3.24 X 10^3	6.05 X 10^3
Sandell's sensitivity (µg cm^{-2})	0.0627836	0.033973
Limit of Detection [LOD] (µg mL^{-1})	0.316709	1.9526
Limit of Quantitation [LOQ] (µg mL^{-1})	0.959725	0.64437
Regression equation[Y*]		
Slope [B]	0.03357	0.044
Intercept[A]	-0.0444	-0.15746
Correlation coefficient [r]	0.9962	0.9930
Relative standard deviation[b]	0.05	0.082

*Y= BX+A, where X is the concentration of the measured solution in µg mL^{-1} and Y is the unit for absorbance. [b]Average of five determinations (concentrations of 5, 15 and 25 µg $mL^{-1}$for PEH and 7.5,12.5 and 17.5 µg mL^{-1} respectively.)

Table 2: Recovery of drugs from solution in presence of a with a 100 fold concentration of various additives used as excipients in formulation.

Excipients	% Recovery ± %RSD[a]	
	PEH[b]	**POC[c]**
Dextrose	99.9 ± 0.4	99.8 ±0.5
Lactose	100.0 ± 0.2	99.9 ± 0.1
Sucrose	99.9 ± 0.4	99.8 ± 0.2
Starch	99.9 ± 0.1	99.7 ± 0.2
Talc	99.8 ± 0.2	99.7 ± 0.5
Magnesium stearate	99.8 ± 0.4	99.8 ± 0.5

[a] Mean ± R.S.D, n=3, [a]mean of three determinations

[b]concentration of PEH used – 5 µg/mL

[c]concentration of POCused – 12.5 µg/mL

Precision studies

The short term precision (intraday precision) of the drugs were evaluated by measuring 5 independent samples at 3 different concentration levels (5.0, 15.0, 25 µg mL^{-1} for Phenylephrine hydrochloride and 7.5, 12.5, 17.5 µg mL^{-1} for Pyridoxine hydrochloride). Similarly the assay for daily precision (interday precision) at the same concentration level was repeated for 5 consecutive

days Table 3. The available pharmaceutical dosage forms of the investigated drugs were analyzed by the proposed method. The precision of the method was checked by taking five replicate measurements. The results obtained by the proposed and the reference methods for the dosage forms were compared statistically by means of F- and t- test and were found not to differ significantly at 95% confidence level. The reliability and accuracy of the proposed method were further ascertained through recovery studies using the standard addition method by adding different amount of standard drugs to the pre analyzed dosage forms such that the cumulative amount after adding the drugs did not exceed their linearity range Table 4**.**

Table 3: Intra day and Inter day precision data of PEH and POC.

Formulation	**Amount taken µg/mL**	**Intra day % Recovery ± %RSD[a]**	**Inter day % Recovery ± %RSD[b]**
PEH	5.0	5.16 ± 0.39	5.10 ± 0.39
	15.0	14.96 ± 0.40	14.98 ± 0.4
	25.0	24.85 ± 0.05	24.9 ± 0.05
POC	7.5	7.43 ± 0.75	7.45 ± 0.7
	12.5	12.38 ± 1.07	12.4 ± 1.0
	17.5	17.54 ± 0.08	17.49 ± 0.07

[a] Mean value of five determinations, [b]Mean of five determinations performed over a period of five days.

Table 4: Analysis of drugs in pharmaceutical formulations.

Formulation studied	**Labeled claimed mg[a]**	**Amount found by proposed method ± SD, mg[a]**	**Reference method ± SD[a]***	**% Recovery by the proposed method[b] ± % RSD**
Frenin(inj)[c]	10mg/mL	9.99 ± 1.21 t = 0.14 F = 1.47	10.1 ± 1.47 **[5]**	99.97 ± 0.12
Benadon[d]	40mg/tab	40.1 ± 0.60 t = 0.94 F = 1.10	39.78 ± 0.57 **[24]**	100.2 ± 0.14

[a]Mean of five determinations ± Standard deviation. n=5; the t- and F-values obtained after comparison to the reference methods, which have the following theoretical values at 95% confidence limit t=2.44 and F=5.05. After adding two different amounts of pure drugs to the fixed concentration of preanalysed pharmaceutical formulations, [c]PEH equivalent to 10mg/mL (Samarth life sciences Pvt. Ltd (unit II), India), [d]POC equivalent to 40mg/tab (piramal laboratories Ltd, India). References inside the brackets are the reported methods given under references**.**

CONCLUSION

The proposed spectrophotometric method for the determination of phenolic drugs is fairly sensitive, simple, and economical with reasonable precision and accuracy. The optical parameters and statistical comparison justify this method for application in routine drug estimation in pure and

dosage forms. Also, the procedures do not involve any critical reaction conditions or tedious sample preparation steps. So, the recommended method is well suited for the assay and evaluation of drugs in pharmaceutical preparation and can also be considered as a general method for the quantification of phenolic drugs.

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