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New spectrophotometric microdetermination of carbapenem antibiotics derivatives in pharmaceutical formulations

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Abstract

A new sensitive spectrophotometric method was developed to determine three carbapenem antibiotics: imipenem, meropenem and ertapenem. The proposed method was based on the formation of the coloured tris(o-phenanthroline)-iron(II) complex (ferroin) [Phen] or Fe (II)-2,2′-bipyridyl complex [Bip] in the reaction of the tested drugs with the corresponding iron (III)-complexes in an acetate pH 4 buffer. The formed coloured complexes showed maximum absorbance at 510 and 520 nm for [Phen] and [Bip], respectively. The reaction conditions, including the pH, reagent concentration, reaction time, temperature and stability of the formed coloured species, were optimized to achieve the highest sensitivity. Linear calibration curves were obtained in the concentration ranges from 0.2 to 10, 0.5 to 10 and 0.5 to 10 $\mu g\,mL^{-1}$ for the aforementioned drugs in the same order. The developed method was successfully applied to determine the investigated carbapenems in their pharmaceutical formulations with average recoveries of 100.8, 99.8 and 99.4% for the Phen method and 98.9, 101.7 and 100.6% for the Bip method for imipenem, meropenem and ertapenem, respectively. A statistical comparison of the results with the reference method showed good concurrence and indicated no significant difference in accuracy or precision.

Keywords: Carbapenem antibiotics; Spectrophotometry; Oxidation reaction; Pharmaceutical analysis

1. Introduction

Carbapenems are β-lactam antibiotics, such as penicillins and cephalosporins, that inhibit bacterial cell wall synthesis by binding to penicillin-binding proteins (PBP) [1,2]. The analogues of carbapenem (imipenem, {(5*R*,6*S*)-6-[(1*R*)-1-hydroxyethyl]-3-({2-[(iminomethyl)amino]ethyl}thio)-7-oxo-1-azabicyclo[3.2.0] hept-2-ene-2-carboxylic acid}), meropenem, ({3-[5-(dimethylcarbamoyl) pyrrolidin-2-yl] sulfanyl-6-(1-hydroxyethyl)-4-methyl-7-oxo-1-azabicyclo[3.2.0] hept-2-ene-2-carboxylic acid), and ertapenem ({(4*R*,5*S*,6*S*)-3-[(3*S*,5*S*)-5-[(3-carboxyphenyl)carbamoyl]pyrrolidin-3-yl]sulfanyl-6-(1-hydroxyethyl)-4-methyl-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid}), which are used in treatment, have an exceptionally broad spectrum of antibacterial activity. Since the late 1970s, when thienamycin was discovered, the next analogues of carbapenem have been introduced into

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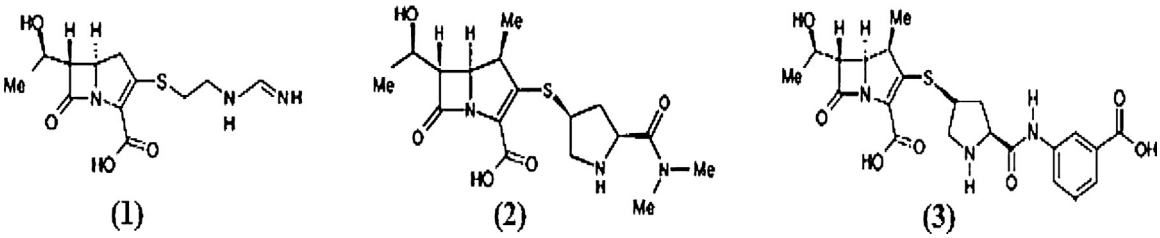

Fig. 1. Chemical structures of imipenem (1), meropenem (2), and ertapenem (3).

therapeutic use, which include imipenem, panipenem, meropenem, ertapenem, biapenem and doripenem. The three carbapenems currently under investigation using the developed method are imipenem, meropenem and ertapenem (Fig. 1).

Several analytical techniques were published for carbapenem analysis [3]. Currently, the most common techniques are chromatographic methods [4–8]. In addition to the chromatographic methods, capillary zone electrophoresis [9,10] and a microbiological assay [11] were also reported. The determination of imipenem and its metabolites in human urine and pharmaceutical formulations using electrochemical methods were reported [12,13]. The electrochemical protocol was performed in phosphate buffer solutions over a pH range of 2.0 to 8.0 using differential pulse polarography, cathodic adsorptive stripping voltammetry, cyclic voltammetry, linear sweep voltammetry and adsorptive stripping voltammetry. The proposed methods have been used for the direct determination of imipenem in spiked human urine and real human-derived urine with good results and should be appropriate for monitoring purposes with a detection limit of 0.28 $\mu g\,L^{-1}$ imipenem.

Spectrophotometric methods were also reported for the determination of carbapenems. Classic UV spectrophotometry has been used to estimate meropenem in powder for injection (wavelength is 298 nm) [14], and first-derivative bivariate procedures have been applied to determine meropenem in the presence of its metabolite (open-ring degradation product) [15]. The UV methods are notably simple, rapid and economical, and they enable drug determination with sufficient reliability.

Drug quality control is a branch of analytical chemistry with a wide impact on public health; thus, the development of reliable, quick and accurate methods for active-ingredient determination is notably important. Spectrophotometric methods are the most commonly used techniques and continue to enjoy wide popularity [16–18]. The common availability of the instrumentation, simplicity of the procedures, and speed, precision and accuracy of the technique make spectrophotometric methods attractive. Redox reactions have been used as the basis to develop simple and sensitive spectrophotometric methods to determine many pharmaceutical compounds [19–21]. In oxidimetric reactions, the most commonly used oxidizing agents is Fe (III), which reduces to Fe (II), followed by complexation with either 1,10-phenanthroline [Phen] or 2,2′-bipyridyl [Bip] with an absorbance at 510 or 520 nm, respectively. The present study was dedicated to investigate the application of these reagents in a new spectrophotometric determination of three carbapenem derivatives (imipenem, meropenem and ertapenem) in their pharmaceutical dosage forms. The proposed methods can be used for quality control analysis, where modern and expensive apparatuses, such as GLC, HPLC and HPTLC, are not available.

2. Experimental

2.1. Materials and reagents

All chemicals were of analytical reagent grade, and double-distilled water was used throughout the experiments. The carbapenem pharmaceutical preparation vials were purchased from the Kingdom Saudi Arabia local drug stores. The pharmaceutical preparations were as follows: Tienam (Tienam*-500 imipenem/cilastain sodium, Merck Sharp & Dohme B.V. Harlem, Netherlands), Meronem (Meronem™, meropenem trihydrte 500 mg, Astra Zeneca UK limited, Macclesfield, Cheshire, Sk 10 2NA, United Kingdom) and Ertapenem (Invanz®, 1-g vials, BN: NE20790, labelled to contain 1 g of ertapenem (equivalent to 1.046 g of ertapenem sodium, 175 mg of sodium bicarbonate). The concentrations of the corresponding active ingredients were estimated according to the official methods [22,24]. Drug stock solutions (100 $\mu g\,m\,L^{-1}$) were always freshly prepared on the day of analysis and stored in a refrigerator to be used within 24 h.

Fe (III)-o-phenanthroline [Phen] reagent [25] was prepared by mixing 0.198 g of 1,10-phenanthroline monohydrate (Fluka, Swiss) with 2.0 mL of 1.0 M HCl and 0.16 g of ferric ammonium sulphate dodecahydrate

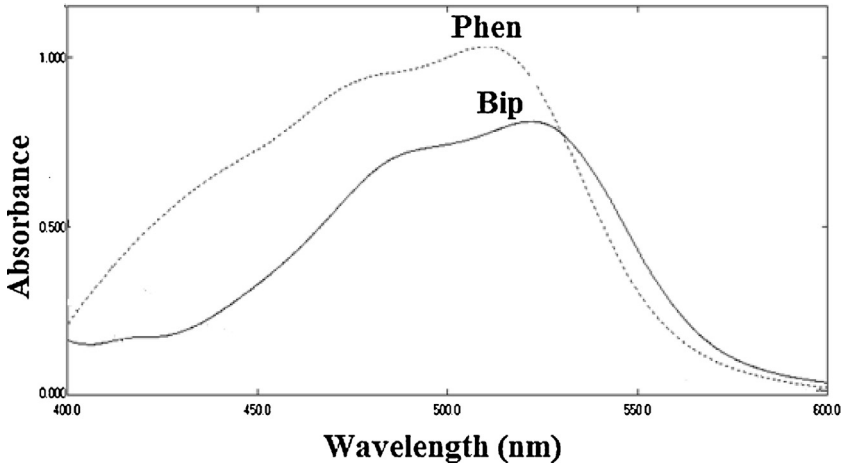

Fig. 2. Absorption spectra of Phen and Bip complexes in presence of 4.0 μg mL^{-1} imipenem.

(Fluka, Swiss) and diluting with bidistilled water to 100 mL. The Fe(III)-bipyridyl [Bip] reagent was prepared by dissolving 0.16 g of 2,2′-bipyridyl in 2.0 mL of 1.0 M HCl and 0.16 g of ferric ammonium sulphate dodecahydrate and diluting with bidistilled water to 100 mL.

2.2. Apparatus

The preliminary spectrophotometric measurements were recorded on a Shimadzu UV-240 spectrophotometer (Kyoto, Japan) with 10-mm light-path cells. A JASCO V-570 (Jasco, Japan) double-beam spectrophotometer (Jasco) was used with 10-mm light-path cells for the absorbance measurements. The software package that accompanied this instrument (Spectra Manager for Windows version 1.21.00) was used for instrument control and data handle, whereas the software Spectra Analysis version 1.24.00 was used to convert to and save the spectra as ASCII-typed files. The pH measurements were performed with a Metrohm 692 pH-metre using a combined electrode. The measurements were performed in a double-jacket thermostated glass cell using a Haake thermosetting circulating water bath with a temperature stability of 70.0 ± 1.0 °C.

2.3. General procedure

Aliquots of different carbapenem standard solutions were transferred into a series of 10-mL calibrated flasks followed by adding 2 mL of [Phen] or [Bip] reagent solution and 2.0 mL of a pH 4.00 acetate buffer solution. The reaction mixture was heated on a water bath at 70 °C for 15 min. After cooling to room temperature, the volume was diluted to the mark with double-distilled water, and the formed coloured complexes were measured at 510 and 520 nm for the [Phen] and [Bip] methods, respectively, against a reagent blank, which was similarly treated. The absorbance values were recorded and plotted against the drug concentration in μg mL^{-1}.

3. Results and discussion

Ferric ions play a prominent role in the spectrophotometric determination of many pharmaceutical drugs by acting as oxidants, whereas a ferric ion is reduced to a ferrous ion in extent equivalent to the drug concentration. The liberated Fe (II) can be determined using selective chelating agents, such as 1,10-phenanthroline and 2,2-bipyridyl [26]. The proposed methods are based on the formation of tris(o-phenanthroline)-iron(II) [Phen] or tris (bipyridyl)-iron(II) [Bip] chelates upon the reduction of the corresponding Fe (III) complexes with different carbapenem derivatives [21,25,27–29] with maximum absorptions at 510 and 520 nm, respectively (Fig. 2). Because of the critical role of the reaction conditions on the analysis performance, comprehensive studies were performed, for example, the effect of the measured wavelength, buffer, concentration and sequence of reagent addition were tested in detail to select the optimal measuring conditions.

3.1. Effect of pH

Different buffer media, such as universal, phosphate, borate, and acetate buffer solutions, were examined to achieve the maximum colour intensity at a fixed carbapenem concentration. The acetate buffer proved to be

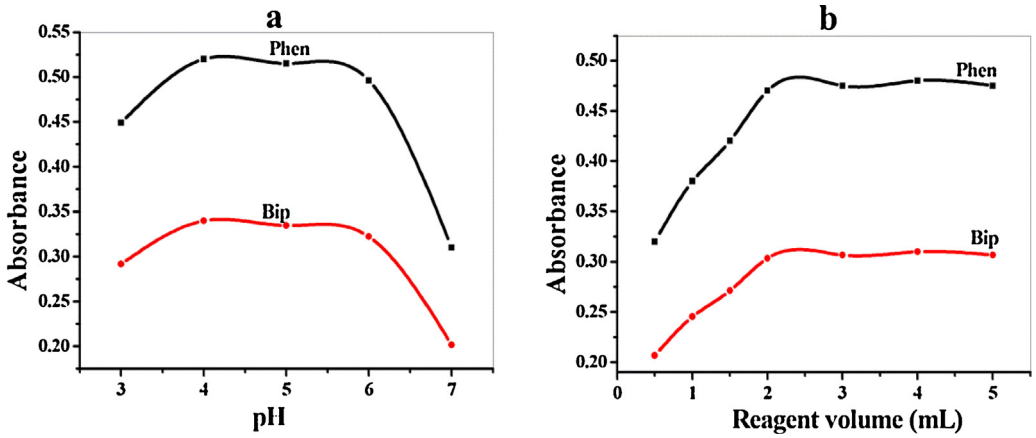

Fig. 3. Effect of pH and reagent concentration on the absorbance of the [Phen] and [Bip] complexes in presence of 4.0 μg mL^{-1} Imipenem.

the most favourable buffer with the highest absorbance of the formed coloured complexes. A pH adjustment was necessary, particularly in an acidic medium, because of the precipitation of ferric ions. The studied pH values were from 3.0 to 8.0, and the optimum pH value was 4.00 ± 0.20 (Fig. 3a). Moreover, a 2.0 ± 0.1 mL buffer solution in the reaction medium was sufficient for complete colour development.

3.2. Effect of the reagent concentration

Different amounts of Phen or Bip reagents were added to the reaction mixture, which contained 4 μg mL^{-1} of imipenium, and the absorbance of the formed coloured complexes was subsequently measured at 510 and 520 nm, respectively. An addition of 2.0 ± 0.2 mL of each reagent was sufficient to obtain the maximum and reproducible absorbance (Fig. 3b). Smaller volumes of the reagents led to incomplete complex formation, whereas higher volumes did not improve the method sensitivity.

3.3. Effect of the reaction temperature and heating time

The effects of the temperature and heating time on the development of the coloured complexes were studied under the optimal pH and reagent concentration conditions. The reaction of imipenem with both reagents slowly proceeded at room temperature and was accelerated by heating (Fig. 4). The maximum absorbance was obtained after heating at 70.0 ± 1.0 °C for approximately 15.0 ± 2.0 min. Further heating caused no appreciable

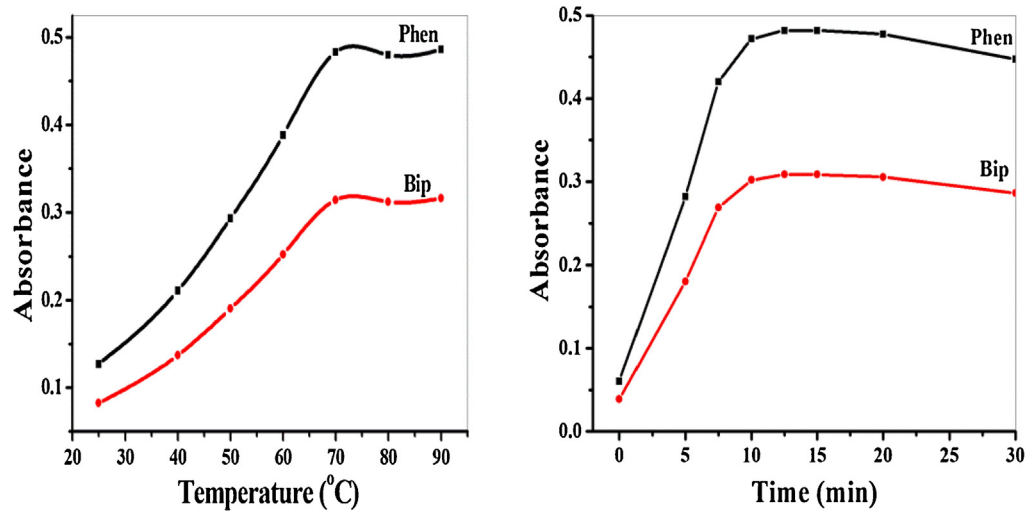

Fig. 4. Effect of temperature and reaction time on the absorbance of [Phen] and [Bip] complexes in presence of 4.0 μg mL^{-1} Imipenem.

Table 1
Characteristics of different calibration graph for carbapenem derivatives by developed Phen and Bip methods.

Parameter	Units	Imipenem		Meropenem		Ertapenem	
		Phen	Bip	Phen	Bip	Phen	Bip
Wavelength	(nm)	510	520	510	520	510	520
Beer's concentration range	($\mu g\,mL^{-1}$)	0.2–10.0	0.2–10.0	0.5–10.0	0.5–10.0	0.5–10.0	0.5–10.0
Ringbom concentration range	($\mu g\,mL^{-1}$)	0.35–9.5	0.33–9.7	1.05–8.7	1.2–8.8	0.5–9.0	0.52–9.4
Detection limits	($\mu g\,mL^{-1}$)	0.064	0.055	0.042	0.074	0.112	0.145
Quantification limits	($\mu g\,mL^{-1}$)	0.211	0.176	0.139	0.244	0.358	0.435
Molar absorptivity	$\times 10^4$ ($L\,mol^{-1}\,cm^{-1}$)	5.60	3.50	7.00	5.75	8.70	6.58
Sandell sensitivity	($\mu g\,cm^{-2}$)	0.0142	0.0128	0.0247	0.01976	0.0227	0.0176
Regression equation	($Y = a + bX$)[a]						
Slope (b)		0.175	0.114	0.1691	0.13708	0.17315	0.12111
S.D. of slope (S_b)		3.76×10^{-3}	2.10×10^{-3}	0.00238	0.00339	0.00648	0.00587
Intercept (a)		5.08×10^{-2}	2.80×10^{-2}	0.05416	0.0346	0.08571	0.08065
S.D. of intercept (S_a)		1.80×10^{-4}	1.00×10^{-3}	0.0238	0.01785	0.0341	0.03086
Correlation coefficient		0.9984	0.9988	0.9979	0.9982	0.9958	0.9930
Calculated t-values (2.57)[b]		1.07	0.70	1.10	0.80	1.03	0.74
Calculated F-test (5.05)[a]		2.37	2.13	2.47	2.22	2.52	2.19

[a] $Y = a + bX$, where Y is the absorbance of the coloured complex and X is the concentration of drug in $\mu g\,mL^{-1}$.
[b] Values in parentheses are the theoretical values for t- and F-value at 95% confidence limits and five degrees of freedom.

change in colour. Moreover, the formed coloured complexes were stable for more than 24 h.

3.4. Method validation

The consistency with Beer's law was studied by measuring the absorbance values of solutions with various drug concentrations. The analytical parameters, such as the molar absorptivity, Sandell's sensitivity, detection limit, quantification limit, slope, intercept, and correlation coefficients, were described (Table 1). The calibrations graphs are described by the regression equation: $Y = a + bX$ (where Y = absorbance, a = intercept, b = slope and X = concentration in $\mu g\,mL^{-1}$), which was obtained using the method of least squares.

The calibration graphs (Fig. 5) were rectilinear in the concentration range of 0.2 to 10 $\mu g\,mL^{-1}$, and the molar absorptivities were 3.50×10^4 to 8.70×10^4. In addition, a detection limit of 0.042 $\mu g\,mL^{-1}$ was obtained for meropenem using the Phen method.

3.5. Accuracy and precision

The accuracy and precision of the methods (within-assay and between assays) were determined at different imipenem concentrations (Table 2). The within-assay precision was assessed by analysing five replicates of each sample as a batch in a single assay run, and the between-assays precision was assessed by analysing the same sample in triplicate in two separate assay runs. The average recoveries were 95.0–104%, and the relative standard deviations (RSD) were less than 1.3% (Table 2). The obtained level of precision was suitable for the quality control analysis of imipenem.

3.6. Interference studies

In pharmaceutical analysis, it is important to test the selectivity of the method for the excipients that are commonly added to pharmaceutical preparations, such as glucose, starch, talc, lactose, and sucrose. The proposed spectrophotometric methods have the advantages that the measurements are performed in the visible region, away from the UV-absorbing interfering

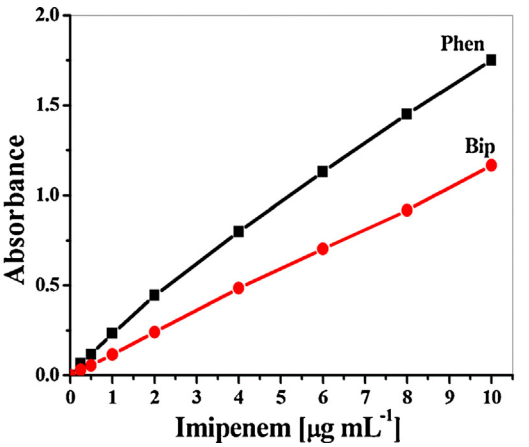

Fig. 5. Spectrophotometric determination of imipenem using [Phen] and [Bip] complexes at the optimum experimental conditions.

Table 2
Accuracy and precision of the proposed methods for analysis of imipenem ($n = 5$).

Method	Taken ($\mu g\,mL^{-1}$)	Found ($\mu g\,mL^{-1}$)					
		Within-assays			Between-assays		
		Found ($\mu g\,mL^{-1}$)	Recovery (%)	RDS (%)	Found ($\mu g\,mL^{-1}$)	Recovery (%)	RDS (%)
Phen	1.0	0.95	95.00	0.88	0.97	97.00	1.25
	3.0	2.86	95.33	0.78	3.12	104.00	0.95
	5.0	4.78	95.60	1.10	4.92	98.40	0.98
	8.0	7.89	98.63	0.95	7.91	98.88	1.25
Bip	1.0	1.01	101.00	0.65	0.99	99.00	0.66
	3.0	3.02	100.66	0.75	2.92	97.33	0.77
	5.0	5.10	102.00	1.05	4.85	97.00	0.96
	8.0	7.93	99.12	0.82	7.88	98.50	0.74

Table 3
Determination of carbapenem in pharmaceutical preparations by the proposed and official method.

Sample	Recovery		
	Phen	Bip	Official method
Tienam (500 mg)	Recovery 100.8 ± 1.0 Variance 0.71 F^* 2.82 t^* 1.93	Recovery 98.9 ± 1.3 Variance 0.59 F 2.53 t 0.55	Recovery 99.6 ± 1.3
Meronem (500 mg)	Recovery 99.8 ± 0.61 Variance 0.55 $F = 1.74$ $t = 0.73$	Recovery 101.7 ± 0.92 Variance 0.49 F 2.03 t 0.92	Recovery 101.0 ± 1.0
Invanz (1.0 g)	Recovery 99.4 ± 0.80 Variance 0.44 F 3.13 t 0.88	Recovery 100.6 ± 0.9 Variance 1.05 F 3.05 t 1.22	Recovery 100.0 ± 1.4

substances that may be present in the dosage forms. Regarding the interference of the common excipients and additives in pharmaceutical formulations (sodium lauryl sulphate, magnesium stearate, starch sodium glycolate, lactose spray dried, carboxymethylcellulose PA 102, talc, titanium dioxide, microcrystalline cellulose, hydroxypropylcellulose and pre-gelatinized starch), no change in the absorbance of the formed complex was observed in the presence of such interferents, which indicates the high selectivity of the proposed methods.

3.7. Analytical applications

The obtained satisfactory validation results made the proposed methods suitable for the routine quality control analysis of carbapenem derivatives (imipenem, ertapenem and meropenem) in their pharmaceutical preparations. The results of the proposed methods were statistically compared with those of the official pharmacopoeia method. The obtained mean values of the labelled amounts were 98.90 and 101.7% using the Phen and Bip methods, respectively, as recorded in Table 3. In the *t*- and *F*-tests, no significant difference was found between the calculated and theoretical values of both the proposed and reported methods at the 95% confidence level [22–24]. These results evidently show that all of the proposed methods can be applied to analyze different carbapenems in their formulations with a comparable analytical performance.

4. Conclusion

The presently proposed method represents simple and sensitive new spectrophotometric measurements of different carbapenem derivatives in their pharmaceutical formulations. The redox reaction of carbapenem using Fe^{3+} and the formation of Fe^{2+}-Phen and Fe^{2+}-Bip complexes were used to develop a simple, accurate, highly

sensitive, and selective spectrophotometric method. The proposed methods can be used in quality control laboratories as alternative methods to the official ones for the routine determination of the aforementioned formulations.

Acknowledgments

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