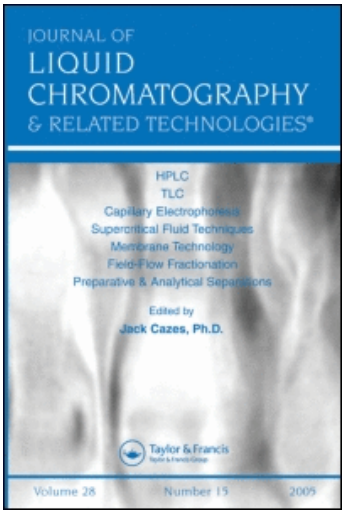

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Göksel Altiokka[a]; Nafiz Öncü Can[a]; Hassan Y. Aboul-Enein[b]
[a] Department of Analytical Chemistry, Faculty of Pharmacy, Anadolu University, Eskisehir, Turkey [b] College of Pharmacy, University of Sharjah, Sharjah, United Arab Emirates

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Determination of Amoxicillin by Flow Injection Analysis using UV-Detection, Potentiometry, and Conductometry in Pharmaceutical Preparations

Göksel Altiokka and Nafiz Öncü Can
Department of Analytical Chemistry, Faculty of Pharmacy, Anadolu University, Eskisehir, Turkey

Hassan Y. Aboul-Enein
College of Pharmacy, University of Sharjah, Sharjah, United Arab Emirates

Abstract: A method for direct determination of amoxicillin in pharmaceuticals using flow injection analysis (FIA) with UV detection adjusted at 228 nm, along with potentiometer and condoptometric methods are described. The best carrier solvent system consisted of an aqueous solution of methanol (10% v/v) with pH 9 pumped at a flow rate of 1 mL · min^{-1}. The limit of detection (LOD) and quantification (LOQ) for FIA was calculated to be 3.3×10^{-7} M (S/N = 3) and 1.0×10^{-7} M (S/N = 10), respectively. Two pharmaceutical formulations containing amoxicillin were studied. In the analysis of the tablets, the RSD values were found to be 1.36 and 1.21 and 1.18 (for AMOKLAVIN®), 1.10 1.16 and 1.14 (for REMOXIL®) for FIA, potentiometer, and conduct metric methods, respectively. The proposed methods were applied to the pharmaceutical tablets and good results were obtained. These proposed methods are simple, fast, accurate, precise, and cost effective for the routine determination of amoxicillin in pharmaceutical preparations.

Keywords: Amoxicillin, Flow injection analysis, Pharmaceutical analysis, Potentiometric analysis, Conductometric analysis

Address correspondence to Hassan Y. Aboul-Enein, College of Pharmacy, University of Sharjah, Sharjah 27272, United Arab Emirates. E-mail: hyaboulenein@yahoo.com

INTRODUCTION

Amoxicillin, [4-Thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid, 6-[[amino-(4-hydroxyphenyl) acetyl] amino]-3,3-dimethyl-7-oxo-, trihydrate [2S-[2α, 5 α, 6β (S*)]]-] (AMX) (Fig. 1) is a wide-spectrum bactericide antibiotic that inhibits bacterial cell wall synthesis. AMX is often used as the first line drug for treating acute otitis media because of its activity against the *H. influenzae*.[1] High molecular weight impurities of amoxicillin are generated easily during protection and storage of the material.[2]

Several analytical methods had been reported based on spectrophotometry,[3–6] HPLC,[7–12] voltammetry,[13] sequential injection analysis,[14] kinetic spectrophotometry,[15] capillary electrophoresis.[16,17]

Flow injection analysis (FIA) is a technique characterized by its versatility, ease of automation, high sampling frequency, and minimum sample treatment prior to injection into the system. The FIA technique found wide applications mainly due to reduction of the analysis time and reagents consumption compared to conventional manual procedures.[18–29] On the other hand, their high sensitivity makes them suitable for the determination of low concentrations of pharmaceuticals in biological fluids when used as detectors in HPLC. They can also optimize the detection of analysis independently from the way the process is occurring in the chromatographic column.[30–37] In addition, potentiometric and conductometric titrations were suitable for the determination of a relatively large amount of the drugs. The apparatus required for making potential and conductance measurements and performing of titrations are generally inexpensive and basically simple in details. For these reasons, the measurements of potential (or pH) and conductance finds wide acceptance in industry as an analytical tool, both in the laboratory and in the process and quality control for routine analyses.[38–40]

The aim of this study is to develop simple, fast and validated methods for the analysis of AMX using FIA, potentiometric and conductometric methods, and the application to the pharmaceutical formulations.

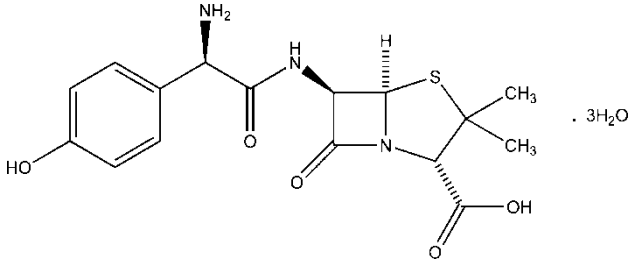

Figure 1. The chemical structure of AMX.

EXPERIMENTAL

Apparatus and Chemicals

WTW Multiline P4 Universal potentiometer-conductometer cabled WTW Sen-Tix 97T combined glass pH electrode and WTW Tetracon 325 conductometric electrode cell (Germany), a Shimadzu Spectrophotometer Model UV 2401 PC (Japan), and quartz cells in the measurement of the absorbance were used.

The HPLC apparatus used was a Model LC 6A pump equipped with a 20 μL manual loop injector, a Model SPD-A10 UV variable wavelength detector, and a Model C-R7A integrator (all Shimadzu, Japan).

Standard AMX (99.0%), REMOXIL® tablets containing 1000 mg active material, and AMOKLAVIN® tablets containing 500 mg active material and potassium clavulanate equivalent to 25 mg clavulanic acid, were kindly supplied from I.E. Ulagay A.S. (Istanbul, Turkey) and Deva Holding A.S. (Istanbul, Turkey), respectively. Other chemicals were of analytical grade from Merck KGaA (Darmstadt, Germany).

Procedures

Flow Injection Analysis

A stock solution of AMX (1×10^{-3} M) was prepared using bidistilled water and the dilutions were made in the range of 1.0×10^{-6}–5.0×10^{-6} M. As the carrier phase an aqueous solution of MeOH (10%, v/v) was used. The buffer solutions were prepared using 1 M CH_3COONa (pH 1–6) and 1 M K_2HPO_4 (pH 7–12), and their pH values were adjusted in the range of 1 and 11 using 2 M HCl or 2 M KOH.

Potentiometry and Conductometry

Standard AMX was weighed, transferred to a beaker, added to 60 mL methanol, and titrated by 0.0998 M NaOH. Buffer solutions of pH 4.87 and 8.05 for pH-meter, 0.01 M KCl for conductometer, were used in the calibration. Both electrodes submerged into the titration solution; potential and conductivity were recorded at the same time of the addition of each titrant volume.

Spectrophotometry

A series of standard AMX dilutions in the concentration range 1.0×10^{-5} and 5.0×10^{-5} M was prepared using 1.0×10^{-3} M stock solution. As the solvent

of AMX, 0.0998 M NaOH was employed. The calibration equation was calculated measuring the absorbance values of the standard solutions at 228 nm.

Application to the Tablets

Twenty tablets were weighed and finely powdered in a mortar. The average weight of one tablet was calculated. For the FIA, a sample equivalent to one tablet was weighed and transferred to a 100 mL calibrated flask, 60 mL methanol was added, magnetically stirred for 20 min., and made up to volume with bidistilled water. A sufficient amount of the solution was pipetted in a tube and it was centrifuged for 10 min. The supernatant was diluted to the predetermined values and injected into the sample loop by means of a syringe.

For potentiometry and conductometry, the powder of the tablets equivalent to an average tablet was weighed, transferred to a beaker, 60 mL methanol was added and stirred for 20 min., then titrated by standard NaOH.

RESULTS AND DISCUSSION

The optimization parameters were determined using a 3×10^{-6} M AMX solution. The solvent system consisted of MeOH and bidistilled water. To investigate the percentage of MeOH, it was varied beginning from 10% to 50% (v/v). It was found that the optimum concentration of MeOH, in view of peak morphology, was 10% (v/v). To determine the optimum flow rate, the flow rate was changed from 0.5 mL $\cdot$ min^{-1} to 3 mL $\cdot$ min^{-1} and the best flow rate was found to be 1 mLL $\cdot$ min^{-1}. When the baseline was reached, another sample was injected. The peak areas versus pH are illustrated in Fig. 2.

As seen in Fig. 2, the peak areas showed significant differences above pH 4 and pH 11. These pH values corresponded to approximately pKa1 and pKa2 values of AMX, respectively.[14] These data were supported by the approach mentioned above. However, the differences were minimum at pH 9, and therefore, a phosphate buffer of pH 9 was chosen as working pH.

The signals for the AMX at concentrations ranging from 1.0×10^{-6}–5×10^{-6} M were obtained under the conditions described above and they are given in Figure 3.

Although the prepared solutions give the same signals during a week time, it is not always possible to obtain the true stability of the molecule. For this aim, HPLC and TLC methods are recommended.

The relationship between area under curve (AUC) against AMX concentration was found to be $AUC = 2.79 \times 10^{10}C\ (M) + 18803.1$, $r = 0.9999$. The limit of detection (LOD) and limit of quantification (LOQ) were calculated to be 3.3×10^{-7} M (S/N = 3) and 1.0×10^{-7} M (S/N = 10), respectively.

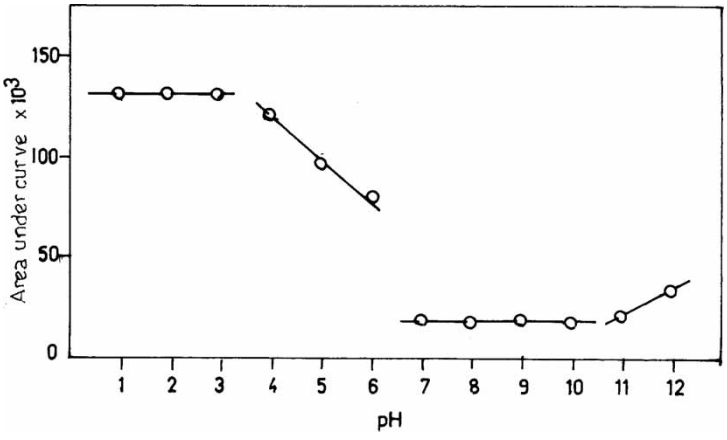

Figure 2. Peak area versus pH for AMX.

Linearity and accuracy in the concentration range of 1.0×10^{-6}–5.0×10^{-6} M were examined, employing intra-day and inter-day studies for the determination of AMX. The results were shown in Table 1.

The titrimetric experiments were realized by submerging the combined glass pH electrode and conductometric cell into the same test solution. After addition of each titrant volume, the variations in the potential and the conductivity were recorded. Plotting the potential and conductivity versus the addition of titrant volume, a well defined S-shape potentiometric and good conductometric graphs were obtained. Both graphs are illustrated in Fig. 4.

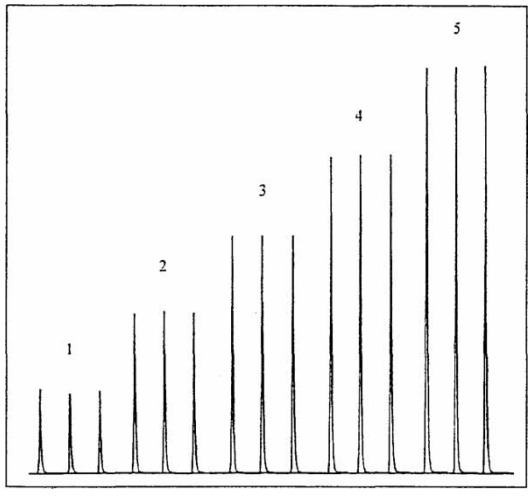

Figure 3. The signals in the 1×10^{-6}–5×10^{-6} M concentration range of AMX with three replicate injections.

Table 1. Linearity and accuracy of FIA method for AMX

Parameters	Intra-day precision (k = 1; n = 8)	Inter-day precision (k = 4; n = 32)
Slope ± SD	$1.81 \times 10^{10} \pm 369$	$1.77 \times 10^{10} \pm 489$
Intercept	3656	3678
Correlation coefficient (r)	0.9996	0.9992
Slope ± CL $_{(p=0.05)}$	$1.81 \times 10^{10} \pm 474$	$1.79 \times 10^{10} \pm 571$

SD: standard deviation CL: confidence limit k: number of the set, n: number of the sample.

At the beginning of titration, the solution of AMX was turbid but its transparency increased gradually around the equivalence point. The equivalence points of AMX were calculated using second derivative curves and the intersection point for the potentiometric and conductometric methods, respectively.

APPLICATION TO THE PHARMACEUTICAL DOSAGE FORMS

The proposed methods were applied to the analysis of AMX in their pharmaceutical tablet formulations. The excipients in the tablets, such as clavulanic

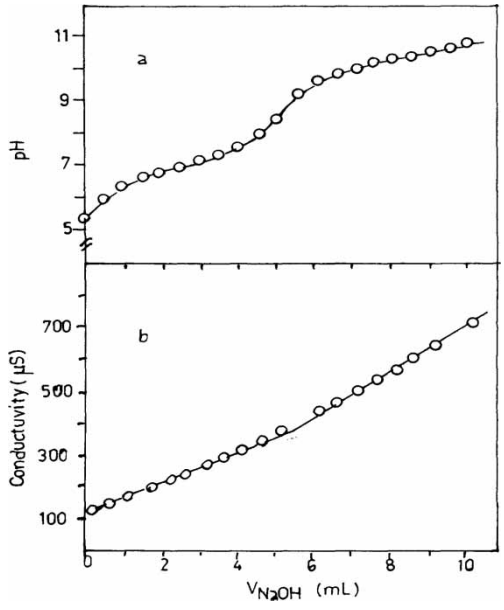

Figure 4. The potentiometric (a) and conductometric (b) curves for titration of AMX (292 mg) with standard NaOH (0.0998 N).

Table 2. Assay results of AMX in tablets[a]

	FIA	Potentiometry	Conductometry	UV
mean	494	492	490	496
n	8	8	8	8
RSD%	0.83	0.98	0.99	0.87
Confidence limit($p = 0.05$)	± 1.86	± 1.14	± 2.17	± 1.92
t-test of significance	1.69	1.81	1.96	$t_{0.05} = 2.14$ (table)
F-test of significance	1.11	1.25	1.29	$F_{0.05} = 4.17$ (table)

[a]Each tablet contains 500 mg of AMX.

acid and titanium dioxide did not interfere in the experiments. The AUC was used for calibration. Spectrophotometry was chosen as a comparison method for the determination of AMX. The absorbance of AMX in 0.0998 M NaOH solution was measured at 228 nm. The relationship between absorbance (A) and concentration (C) was found to be: $A = 10039.8\,C\ (M) - 4.79 \times 10^{-3}$; $r = 0.9999$. The results for these analytical methods are presented in Table 2.

It was observed that the differences among the methods are insignificant at the 95% probability level (F- and t-test). In conclusion, the proposed methods in this study are simple, accurate, precise, rapid, and can be used for routine analysis of AMX in its pharmaceutical dosage forms

REFERENCES

1. *United States Pharmacopeia (USP # 24 NF 19)*; United States Pharmacopeial Convention, Mack Printing Co.: Easton, PA, 2000, 129.
2. Shan-Ying, C.; Chang-Qin, H.; Ming-Zhe, X. Chromatographic determination of high-molecular weight impurities in amoxicillin. J. Pharm. Biomed. Anal. **2003**, *31* (3), 589–596.
3. Salem, H.; Saleh, G.A. Selective spectrophotometric determination of phenolic β-lactam antibiotics. J. Pharm. Biomed. Anal. **2002**, *28* (6), 1205–1213.
4. Mohamed, G.G. Spectrophotometric determination of ampicillin, dicluxacillin, flucloxacillin, and amoxicillin antibiotic drugs: ion-pair formation with molybdenum and thiocyanate. J. Pharm. Biomed. Anal. **2001**, *24* (4), 561–567.
5. Salem, H. Selective spectrophotometric determination of phenolic β-lactam antibiotics in pure forms and in their pharmaceutical formulations. Anal. Chim. Acta. **2004**, *515* (2), 333–341.
6. Amin, A.S.; Moustafa, M.E.; Issa, Y.M. Spectrophotometric microdetermination of ampicillin and amoxicillin with picric and picramic acids. Microchem J. **1994**, *50* (1), 6–13.
7. Hoizey, G.; Lamiable, D.; Frances, C.; Trenque, T.; Kaltenbach, M.; Denis, J.; Millart, H. Simultaneous determination of amoxicillin and clavulanic acid in

human plasma by HPLC with UV detection. J. Pharm. Biomed. Anal. **2002**, *30* (3), 661–666.
8. Sorensen, L.K.; Snor, L.K.; Elkaer, T.; Hansen, H. Simultaneous determination of seven penicillins in muscle, liver and kidney tissues from cattle and pigs by a multiresidue high-performance liquid chromatographic method. J. Chromatogr. B Biomed. Sci. Appl. **1999**, *734* (2), 307–318.
9. Mascher, H.M.; Kikuta, C. Determination of amoxicillin in human serum and plasma by high-performance liquid chromatography and on-line postcolumn derivatisation. J. Chromatogr. A **1998**, *812* (1–2), 221–226.
10. Chulavatnatol, S.; Charles, B.G. High-performance liquid chromatographic determination of amoxicillin in urine using solid-phase, ion-pair extraction and ultraviolet detection. J. Chromatogr. B Biomed. Sci. Appl. **1993**, *615* (1), 91–96.
11. Haginaka, J.; Wakai, J. Liquid chromatographic determination of amoxicillin and its metabolites in human urine by postcolumn degradation with sodium hypochlorite. J. Chromatogr. B Biomed. Sci. Appl. **1987**, *413*, 219–226.
12. Haginaka, J.; Yasuda, H.; Nakagawa, T.U. High-performance liquid chromatographic assay of clavulanate in human plasma and urine by fluorimetric detection. J. Chromatogr. B Biomed. Sci. Appl. **1986**, *377*, 269–277.
13. Uslu, B.; Biryol, I. Voltammetric determination of amoxicillin using a poly (vinyl imidazole) modified carbon paste electrode. J. Pharm. Biomed. Anal. **1999**, *20* (3), 591–598.
14. Pasamontes, A.; Callao, M.P. Determination of amoxicillin in pharmaceuticals using sequential injection analysis and multivariate curve resolution. Anal. Chim. Acta. **2004**, *515* (1), 159–165.
15. Belal, F.; El-Kerdawy, M.M.; El-Ashry, S.M.; El-Wasseef, D.R. Kinetic spectrophotometric determination of ampicillin and amoxicillin in dosage forms. Farmaco **2000**, *55* (11–12), 680–686.
16. Hernández, M.; Borrull, F.; Calull, M. Determination of amoxicillin in plasma samples by capillary electrophoresis. J. Chromatogr. B Biomed. Sci. Appl. **1999**, *731* (2), 309–315.
17. Li, Y.M.; van Schepdael, A.; Zhu, Y.; Roets, E.; Hoogmartens, J. Development and validation of amoxicillin determination by micellar electrokinetic capillary chromatography. J. Chromatogr. A **1998**, *812* (1–2), 227–236.
18. Al-Momani, I.F.; Awni, I.; Khalil, H.S.; Esmadi, F. Spectrophotometric determination of cefadroxil in drug formulations using flow injection analysis. Anal. Lett. **1999**, *32* (15), 2977–2988.
19. Danet, A.F.; Cheregi, M.C.; Badea, M.; Radu, A.; Aboul-Enein, H.Y. Automated flow injection analysis device and methods for determination of chlorine in drinking waters. Rev. Chim. Bucharest **2003**, *54* (1), 3–6.
20. Stefan, R.I.; van Staden, J.F.; Mulaudzi, L.V.; Aboul-Enein, H.Y. On-line simultaneous determination of S- and R-perindopril using amperometric biosensors as detectors in flow systems. Anal. Chim. Acta. **2002**, *467* (1–2), 189–195.
21. Stefan, R.I.; van Staden, J.K.F.; Aboul-Enein, H.Y. Design and use of electrochemical sensors in enantioselective high throughput screening of drugs. A minireview. Comb. Chem. High-Through. Screening **2000**, *3* (6), 445–454.
22. Danet, A.F.; Badea, M.; Aboul-Enein, H.Y. Flow injection system with chemiluminometric detection for enzymatic determination of ascorbic acid. Luminescence **2000**, *15* (5), 305–309.
23. Aboul-Enein, H.Y.; Stefan, R.I.; van Staden, J.F.; Zhang, X.R.; Garcia-Campana, A.M.; Baeyens, W.R.G. Recent developments and applications of chemiluminescence sensors. Crit. Rev. Anal. Chem. **2000**, *30* (4), 271–289.

24. Aboul-Enein, H.Y.; Stefan, R.I.; van Staden, J.F. Chemiluminescence-based (bio) sensors—An overview. Crit. Rev. Anal. Chem. **1999**, *29* (4), 323–331.
25. Stefan, R.I.; van Staden, J.F.; Aboul-Enein, H.Y. Electrochemical sensor arrays. Crit. Rev. Anal. Chem. **1999**, *29* (2), 133–153.
26. Stefan, R.I.; van Staden, J.K.F.; Aboul-Enein, H.Y. Determination of S-perindopril using a flow injection system with an amperometric biosensor. Sens. Actuators B: Chem. **1999**, *54* (3), 261–265.
27. van Staden, A.F.; Stefan, R.I.; Aboul-Enein, H.Y. Amperometric biosensor based on D-aminoacid oxidase for the R-perindopril assay **2000**, *367* (2), 178–180.
28. Gubitz, G.; Schmid, M.G.; Silviaeh, H.; Aboul-Enein, H.Y. Chemiluminescence flow-injection immunoassays. Crit. Rev. Anal. Chem. **2001**, *31* (3), 167–174.
29. Gubitz, G.; Schmid, M.G.; Silviaeh, H.; Aboul-Enein, H.Y. Chemiluminescence flow-injection immunoassays. Crit. Rev. Anal. Chem. **2001**, *31* (2), 141–148.
30. Gonzalez, F.; Tarin, P.; Maspoch, S.; Blanco, M. Flow injection amperometric determination of pharmaceuticals. Arch. Pharm. **1988**, *321* (10), 725–728.
31. Altiokka, G.; Atkosar, Z.; Can, N.O. The determination of levofloxacin by flow injection analysis using UV detection, potentiometry, and conductometry in pharmaceutical preparations. J. Pharm. Biomed. Anal. **2002**, *30* (3), 881–885.
32. Can, N.O.; Tuncel, M.; Aboul-Enein, H.Y. Determination of nabumetone in pharmaceutical formulation by flow injection analysis (FIA) with UV-detection. Pharmazie **2003**, *58* (1), 22–24.
33. Can, N.O.; Altiokka, G. Determination of azapropazone in its pharmaceutical form by HPLC and flow injection analysis. J. Liq. Chromatogr. & Rel. Techol. **2005**, *28* (2), 857–869.
34. Can, N.O.; Altiokka, G.; Aboul-Enein, H.Y. Determination of cefuroxime axetil in tablets and biological fluids using liquid chromatography and flow injection analysis. Anal.Chim Acta. **2006**, *576* (2), 246–252.
35. Altiokka, G.; Altiokka, M. Flow injection analysis of amLodipine using UV-detection. Pharmazie **2002**, *57* (7), 500–500.
36. Altiokka, G.; Kircali, K. Simple method of paroxetine determination using a single channel FIA with no in-line reaction process. Anal. Sci. **2003**, *19* (4), 629–631.
37. Altiokka, G.; Atkosar, Z. Flow injection analysis of doxazosin mesylate using UV-detection. J. Pharm. Biomed. Anal. **2002**, *27* (5), 841–844.
38. Swarbric, J.; Boylan, J.C. *Encyclopedia of Pharmaceutical Technology*; Marcel Dekker Inc.: NY, 1995, Vol. 12, 347.
39. Bauer, H.H.; Christian, G.D.; O'Reilly, J.E. *Instrumental Analysis*; Allyn & Bacon Chemistry Series: Boston, 1978, 117.
40. Aboul-Enein, H.Y.; El-Fatatry, H.M.; Lotfi, E.A. Conductimetric determination of methimazole. Pharmazie **1980**, *35* (10), 604–605.

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