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DEVELOPMENT OF A METHOD FOR THE DETERMINATION OF AMOXICILLIN IN CAPSULES BY POTENTIOMETRIC TITRATION

DESENVOLVIMENTO DE UM MÉTODO PARA A DETERMINAÇÃO DE AMOXICILINA EM CÁPSULAS POR TITULAÇÃO POTENCIOMÉTRICA

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ABSTRACT

This work presents the determination of amoxicillin (H_3Amox^+) in capsules by potentiometric titration. An optimized method was done with a standard amoxicillin solution that was a title with NaOH solution. The initial pH was 4.35. As expected, the first equivalence point was not observed, because the fraction of H_3Amox^+ was about 2.4%. Thus, HCl solution was added to the system to protonate the zwitterion $H_2Amox^{\pm}$ (98%), and then visualize the first equivalence point. Two inflections were observed, the first one of the neutralization of H_3Amox^+ e HCl and the second one of the neutralization of the zwitterion. The equivalent volumes were estimated using the first derivative method, and the volume variation (ΔV) between the equivalent volumes was used to determine the amoxicillin. The results showed 99% of accuracy and coefficient of variation of 7.2%.

RESUMO/RESUMEN

Este trabalho apresenta a determinação de amoxicilina (H_3Amox+) em cápsulas por titulação potenciométrica. A otimização do método foi feita com uma solução padrão de amoxicilina que foi titulada com uma solução de NaOH. O pH inicial foi 4.35. Como esperado, o primeiro ponto de equivalência não foi observado porque a fração de H_3Amox^+ era de 2,4%. Então, uma solução de HCl foi adicionada ao sistema para promover a protonação do zwitterion $H_2Amox^{\pm}$ (98%) e visualizar o primeiro ponto de equivalência. Duas inflexões foram observadas, a primeira referente à neutralização do H_3Amox^+e HCl e a segunda referente à neutralização do zwitterion. O volume de equivalência foi estimado usando o método da primeira derivada e a variação de volume (ΔV) entre os volumes de equivalência foi usada para determinar a amoxicilina. Os resultados mostraram uma exatidão de 99% e coeficiente de variação de 7,2%.

1. INTRODUCTION

Amoxicillin, (2S,5R,6R)-6-[[(2R)-2-amino-2-(4-hydroxyphenyl)acetyl]amino]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid (Figure 1), is a β-lactam antibiotic member of the same family as penicillin. This antibiotic has a broad spectrum against gram-positives and gram-negatives bacteria (Caiaffa et al., 2002; Gomes et al., 2010), it is one of the most used antibiotics for the treatment humans, applied also in the veterinary sector (Pan et al., 2008).

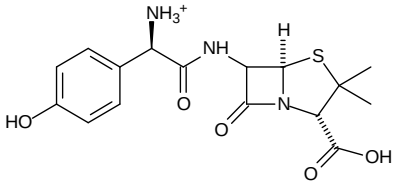

Figure 1 - Chemical Specie totally protonated Amoxicillin structure formula

As a veterinary medicinal product, the amoxicillin is used for the treatment of bacterial infection on the skin, urinary, gastrointestinal and respiratory. In human context, it is used to treat bronchitis, pneumonia, tonsillitis among others infections, and amoxicillin has its due emphasis because great power of absorption following oral administration and its availability to be 95%, approximately (Den Berghe et al., 2011). Amoxicillin usually is combined with potassium clavulanate that functions as enzyme inhibitor by competition a mechanism based β-lactamase, which this enzyme is responsible for inactivates penicillins, improving amoxicillin efficiency (Bejjani et al., 2016).

Many analytical procedures have been reported for the determination of amoxicillin. M.A.M. Silva *et al.*, (2012) determined amoxicillin in pharmaceutical suspension formulations based on transflectance near infrared (NIR) measurements and partial least squares (PLS) multivariate calibration (Silva et al., 2012). Eid and contributors (2016) reported the determination of penicillin traces to verify possible contamination of amoxicillin in non-penicillin pharmaceutical drug product using ultra performance liquid chromatography (UPLC) (Eid et al., 2016). A procedure based on the reaction of amoxicillin with nitroaromatics for determination of amoxicillin in pure form and in pharmaceutical preparations was described by Amin et al. (1994a). Dhoka and contributors (2010) developed and validated a method using high performance thin layer chromatographic (HTPLC) for determination of amoxicillin trihydrate and bromhexine hydrochloride in combined capsule dosage form (Dhoka et al., 2010). Besides these methods previously described, several spectrophotometric (Amin et al., 1994b; Devani et al., 1992; Nagaralli et al., 2002; Qureshi et al., 1999; Saleh et al., 1996; Walash et al., 1994) and chromatographic (Atici et al., 2017; Beg et al., 2012; Rele and Mali 2013) methods have also been related for amoxicillin analysis.

Liquid chromatographic method for analysis of amoxicillin in pharmaceutical dosage has been recommending by US and Indian Pharmacopoeias, which the methods suggested the use of octadecyl silane chemically bonded to porous silica, using a mobile phase of acetonitrile: solvent mixture (96:4) with detector 230 nm. Indian Pharmacopoeia has also recommended potentiometric methods for the analysis of amoxicillin in pharmaceutical dosage. The method consisting of a mixture solution of drug in borate buffer pH 9.0, acetic anhydride, sodium hydroxide, acid nitric and acetate buffer pH 4.6 titrated with mercuric nitrate. Platinum or mercury indicator electrode and mercury-mercurous sulphate reference electrode have used in this potentiometric titration (Kaur et al., 2011). This potentiometric titration method requires the use of many reagents in addition to using mercuric nitrate as titrant being hazardous to analyst health.

Thus, this work proposed a potentiometric titration of an aqueous solution of amoxicillin in which had its pH adjusted to about 3.0 a solution of HCl, titrated with standardized NaOH solution. The method of amoxicillin determination by potentiometric titration that it is a promising technique for being cheap and easy manipulation.

2. Material and Methods

Chemicals

Sodium hydroxide was purchased from Impex, amoxicillin trihydrate standard was purchased from Sigma-Aldrich, hydrochloride acid was purchased from Alphatec and potassium biphthalate was purchased from Vetec. Deionized water from a Milli-Q system was used to prepare all aqueous solutions this study.

Samples

Amoxicillin trihydrate drug was purchased from a drugstore in the city of Viçosa, Minas Gerais state and determined by the proposed method. All content of the capsule was transfer to the beaker and dissolved in 500 mL of water. The solution was maintained with a vigorous mechanical stirring for 10 minutes. Then, the solution was filtered, to remove the excipient contained in the medicinal product, and the filtered was quantitatively transferred to the volumetric flask.

Potentiometric titration

Potentiometric titration was performed with a pH meter (LAB 1000 – MP 210) and combined glass reference electrode, Ag/AgCl intern reference. In a beaker, 100 mL of an aqueous solution of standard amoxicillin (1.37×10^{-3} mol L^{-1}) had its pH adjusted to about 3.0 by 40 µL a solution of HCl (1.00 mol L^{-1}). The system was kept under constant stirring at room temperature. The solution was titrated with NaOH solution (0.01 mol L^{-1}), previously standardized, adding 1.0 mL increments up to 60 mL. All analyses were

conducted in triplicate on three different days. Posteriorly, potentiometric titration described above also was performed with samples of amoxicillin capsules.

Comparative procedures

As comparative procedures to validate the proposed procedure were used spectrophotometric and chromatographic methods. The UV/Vis Molecular Absorption Spectrophotometer used was a Thermo Scientific Evolution Array, composed of a quartz cell of the 1.0 cm optical path and the amoxicillin standard solutions spectra were obtained by scanning from 200 to 300 nm. Various standard solutions were prepared in the concentration range of 2.74×10^{-5} to 2.74×10^{-4} mol L^{-1} and the reading were realized on 272 nm wavelength. The amoxicillin drug solution was diluted 10 times to be analyzed in the spectrophotometer. In the chromatographic method, the amoxicillin was monitored by High-Performance Liquid Chromatography (HPLC-UV) (Shimadzu - LC 20AT) with a UV-Vis detector (Shimadzu SPD 20A) with a C18 column (4.6 × 150 mm, 5.0 µm). Mobile phase used in isocratic elution mode consisted of acetonitrile:phosphoric acid 0.2% (50:50, v/v) at flow rate of 1.0 mL min^{-1}. The injection volume of sample was 20 µL and the UV-Vis detector set at a 272 nm wavelength. The concentrations were estimated using the analytical curves from 2.74 x 10^{-5} up to 2.74 × 10^{-4} mol L^{-1}.

3. RESULTS

Potentiometric Titration

Firstly, the NaOH solution was standardized in replicate using potassium phthalate and was found a concentration of 8.46×10^{-3} mol L^{-1}. In sequence, the amoxicillin standard solution (1.37×10^{-3} mol L^{-1}) was titrated with the NaOH solution (8.46×10^{-3} mol L^{-1}). From the data obtained, the titration curve of amoxicillin system was made (Figure 2). As can be seen, there is an equivalence point close to 20 mL and a slight increasing on the pH.

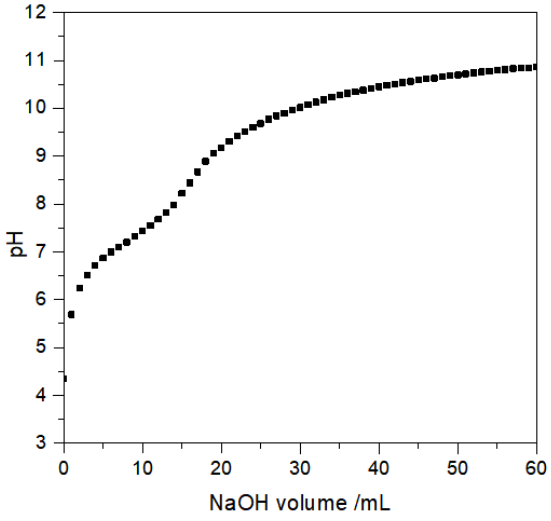

Figure 2. Titration curve of 100 mL amoxicillin standard solution (1.37×10^{-3} mol L^{-1}) with NaOH solution (8.46×10^{-3} mol L^{-1})

The simulation of titration of the fully protonated species (H_3Amox^+) was done for evaluation of this system using the TitGer electronic spreadsheet (Oliveira et al., 2007). The simulation allows observing the potentiometric curve and the behavior of equilibrium fraction (α) of each species of Bronsted's acid-base system of amoxicillin.

It was observed there would be two equivalence points. As can be seen in Figure 3, the first equivalence point is the fully protonated specie H_3Amox^+ being consumed ($\alpha_0\rightarrow0$), in this condition there is a maximum amount of zwitterion ($\alpha_1\rightarrow1$), which it would be almost consumed on second equivalence point ($\alpha_1\rightarrow0$). The third equivalence point, associated with the third proton, does not be observed, because pKa_3 is close to the final pH of the system, in a region with excess hydroxyls (pseudobuffer region) (Bates 1954).

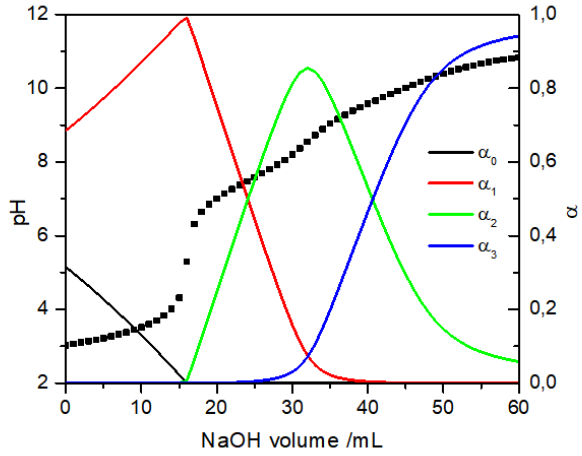

Figure 3. Overlapping the equilibrium fractions (α) on simulated titration curve of amoxicillin with NaOH obtained with electronic worksheet TitGer

Comparing the titration curve obtained experimentally (Figure 2) with a curve obtained by simulation (Figure 3), it can be observed that the initial pH was 4.35 and was not around 3.0. At this initial pH equal 4.35, according to species distribution diagram (Figure 4), the zwitterion is the predominant species with about 99% analytical concentration of the system. Thus, the titration obtained refers to the zwitterion and not to fully protonated species H_3Amox^+.

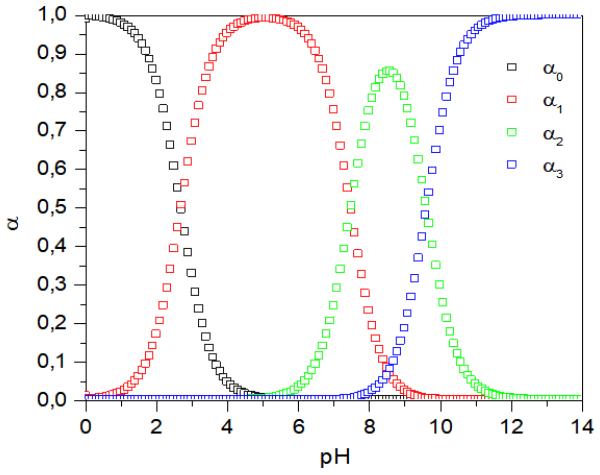

Figure 4. Species distribution diagram of amoxicillin. Graphic generated from electronic worksheet TitGer.

Therefore, to observe the first equivalence point referring to first species H_3Amox+, 40 µL of HCl solution 1.00 mol L^{-1} was added to ensure protonation of amoxicillin. The results are shown in Figure 5. As can be seen, the two equivalence points are present on the curve. In addition, it is possible to estimate the pKa2 value in the mean volume between the equivalence points (16.00 mL), between pH 7.09 and 7.21. This value is reasonably close to value described in the literature (Goddard et al.; 1996).

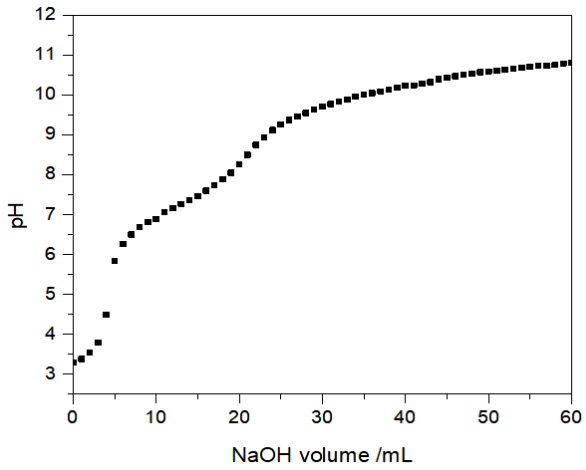

Figure 5. Titration curve of 100 mL amoxicillin standard solution (1.37 x 10^{-3} mol L^{-1}) and 40 µL HCl solution (1.00 mol L^{-1}), with NaOH solution (8.46 x 10^{-3} mol L^{-1})

For a better understanding of titration steps, one can also analyze the species distribution diagram of the system (Figure 4) and the buffer capacity curve versus pH (Figure 6). As can be observed, the maximum points are the system region where there is a maximum buffer region, which it is approximately close to pKa values, that is, 2.7, 7.5 and 9.5, besides to the pseudo buffer regions.

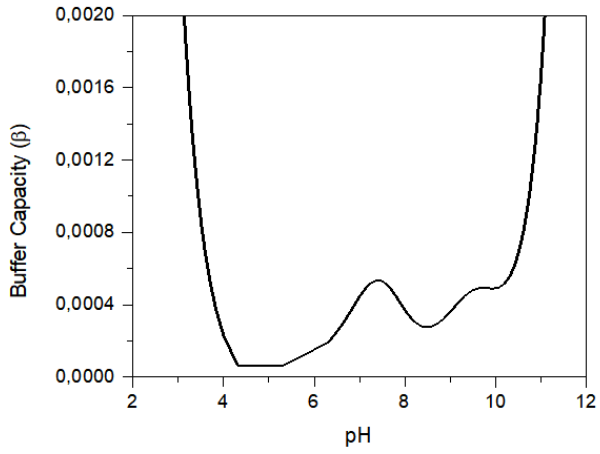

Figure 6. Buffer capacity versus pH to amoxicillin system. Graphic generated from electronic worksheet TitGer

At the beginning of titration, the first equilibrium is the most important referring to a carboxylic group, because it is the strongest acid in the amoxicillin functional groups.

$$H_3Amox^{+}_{(aq)} \leftrightharpoons H^{+}_{(aq)} + H_2Amox^{\pm}_{(aq)} \quad pKa_1 = 2.69 \qquad (1)$$

With the addition of titration, there is a decrease in the concentration of fully protonated species H_3Amox^{+}, forming the zwitterion specie $H_2Amox^{\pm}$. When α_0 tends to zero ($\alpha_0 \rightarrow 0$), at pH close to 4.0, there is in the solution only zwitterion ($\alpha_1 \rightarrow 1$) and the buffer capacity is practically despicable. Thus, in this case, with small additions of NaOH to solution results in an increase in pH. After the first equivalence point with the addition the NaOH, the second equilibrium becomes more important and the solution pH reach the second buffer region, close to pKa_2.

$$H_2Amox^{\pm}_{(aq)} \leftrightharpoons H^{+}_{(aq)} + HAmox^{-}_{(aq)} \quad pKa_2 = 7.49 \qquad (2)$$

In the second equivalence point, there is a smaller buffer capacity at pHs between 8-9 (Figure 6), duo the presence of species $H_2Amox^{\pm}$ and $HAmox^{-}$ in the system. In addition, species $Amox^{2-}$ begins to appear in this pH range. Thus, the pH jump at this point was not very pronounced. How buffer capacity increases in high pH values because is a pseudo-buffer region (in this case, hydroxyls excess), it is not possible observe third equivalence point, because the specie $HAmox^{-}$ is not fully consumed, that is, its equilibrium fraction does not tends to zero.

Determination of amoxicillin concentration by potentiometric titration

The equivalence volumes of amoxicillin system were obtained by first derivative method (Figure 6). When added HCl to the system the pH was changed to around 3.10 and in this pH the equilibrium fraction of more protonated species (H_3Amox^{+}) is 0.28. Therefore, first equivalence volume is associated with the neutralization of this specie (H_3Amox^{+}). Then, from the experimental results obtained the equilibrium fraction found was 0.28 and a coefficient of variation of 9.67%. Used the difference between two equivalence points, associated to stoichiometric ratio equal to one. The mean concentration obtained was 1.33×10^{-3} mol L^{-1}. The method obtained showed good accuracy (99%) and a coefficient of variation of 3.68%.

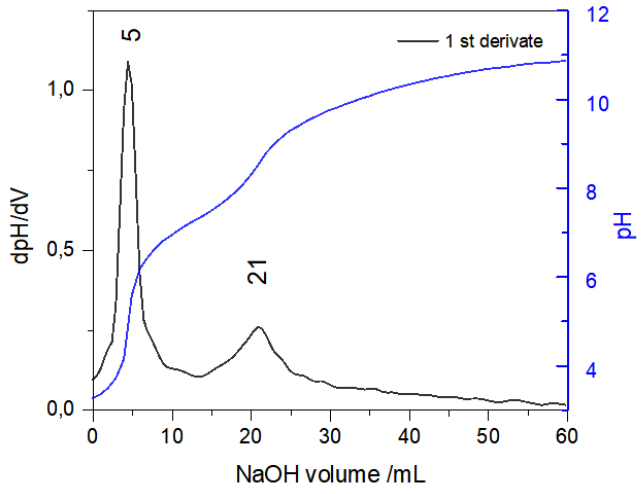

Figure 6. First derivative of potentiometric titration of 100 mL of amoxicillin standard solution (1.37×10^{-3} mol L^{-1}) and 40 µL HCl solution (1.00 mol L^{-1}), with NaOH solution (8.46×10^{-3} mol L^{-1})

After the method optimization to the determination of amoxicillin by potentiometric titration, the titration proceeded with samples of amoxicillin capsules. Titration

curve obtained was similar to standard titration curve with hydrochloric acid added in solution and the difference of equivalent volumes (ΔV) was used and obtained by first derivative method. The mean concentration obtained was 464 mg/capsule and a coefficient of variation of 0.22%.

Comparative procedures

Therefore, to verify the method veracity were used conventional analytical techniques as Spectrophotometry of Molecular Absorption UV-Vis and High Performance Liquid Chromatography, obtaining an average content (and coefficient of variation) of 445 (2.36) and 438 (1.50) mg/capsule, respectively. Likewise, to results obtained by potentiometric titration, the amoxicillin content found in the amoxicillin capsule were lower than described by the manufacturer (500 mg). Analysis of variance (ANOVA) at a 95% confidence level was applied was applied to the results. This analysis indicated that there was not statically significant difference between the amoxicillin content obtained by different techniques. It is important to mentioning that a repeatability of the potentiometric titration was inferior to the other techniques.

Conclusions

This work proposed a method of amoxicillin determination by potentiometric titration. The first step consisted in the adequacy of method of analysis for an experimental proposal from a standard solution of amoxicillin. Then, the optimized method was applied to amoxicillin solution from capsules. The results obtained were compared with conventional techniques such as Liquid Chromatography and Molecular Absorption Spectrophotometry. The results demonstrated that the method is accurate, since that statistically equal results were obtained between the different techniques. The potentiometric method proposed to determination of amoxicillin in commercial capsules is simple, accurate and low cost.

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REFERENCES

AMIN, A. S.; EL-ANSARY, A. L.; ISSA, Y. M. Colorimetric determination of amoxicillin in pure form and in pharmaceutical preparations. **Talanta**, v. 41, n. 5, p. 691–694, may. 1994a.

AMIN, A. S.; MOUSTAFA, M. E.; ISSA, Y. M. Spectrophotometric Microdetermination of Ampicillin and Amoxicillin with Picric and Picramic Acids. **Microchemical Journal**, v. 50, n. 1, p. 6–13, aug. 1994b.

ATICI, E. B.; YAZAR, Y.; AĞTAŞ, Ç.; RIDVANOĞLU, N.; KARLIĞA, B. Development and validation of stability indicating HPLC methods for related substances and assay analyses of amoxicillin and potassium clavulanate mixtures. **Journal of Pharmaceutical and Biomedical Analysis**, v. 136, p. 1–9, mar. 2017.

BATES, R. G. Electrometric pH Determination, John Willey and Sons, New York, 1954.

BEG, S.; KOHLI, K.; SWAIN, S.; HASNAIN, M. S. Development and Validation of RP-HPLC Method for Quantitation of Amoxicillin Trihydrate in Bulk and Pharmaceutical Formulations Using Box-Behnken Experimental Design. **Journal of Liquid Chromatography & Related Technologies**, v. 35, n. 3, p. 393-406, 2012.

BEJJANI, A.; ROUMIÉ, M.; AKKAD, S.; EL-YAZBI, F.; NSOULI, B. Simultaneous quantification of amoxicillin and potassium clavulanate in different commercial drugs using PIXE technique. **Nuclear Instruments and Methods in Physics Research Section B: Beam Interactions with Materials and Atoms**, v. 371, p. 392–395, mar. 2016.

CAIAFFA, M. C.; OLIVEIRA, D. L.; PINHEIRO, V. A.; GUERRA, M. O.; PINHO, J. J. R. G. Estudo biofarmacotécnico de cápsulas de amoxicilina. Análise comparativa de produtos industrializados e magistrais. Cinética de dissolução. **Revista Lecta**, Bragança Paulista, SP, v. 20, n. 1, p.77–90, 2002.

DEN BERGHE, H. V.; GARRIC, X.; VERT, M.; COUDANE, J. New amoxicillin-poly(lactic acid)-based conjugates: synthesis and in vitro release of amoxicillin. **Polymer International**, v. 60, n. 3, p. 398–404, mar. 2011.

DEVANI, M. B.; PATEL, I. T.; PATEL, T. M. Spectrophotometric determination of amoxycillin and its dosage forms. **Journal of Pharmaceutical and Biomedical Analysis**, v. 10, n. 5, p. 355–358, may. 1992.

DHOKA, M. V.; GAWANDE, V. T.; JOSHI, P. P. HPTLC determination of amoxicillin trihydrate and bromhexine hydrochloride in oral solid dosage forms. **Journal of Pharmaceutical Sciences and Research**, v. 2, n. 8, p. 477–483, 2010.

EID, M. A.; YUSOF, N. A.; FARUQ, M.; ABDULLAH, J.; SULAIMAN, Y. Quantitative measurement of amoxicillin in Ibuprofen tablets using UPLC. **Measurement**, v. 93, p. 465–472, nov. 2016.

GODDARD, A. F.; JESSA, M. J.; BARRET, D. A.; SHAW, P. N.; CEDERBERG, C.; SPILLER, R. C. Effect of Omeprazole on the Distribution of Metronidazole, Amoxicillin, and Clarithromycin in Human Gastric Juice. **Gastroenterology**, v. 111, n. 2, p. 358–367, aug. 1996.

GOMES, M. L. P. C.; SOUZA, S. V. C.; Validação de método para determinação de resíduos de amoxicilina aplicado à validação de limpeza em indústria farmacêutica de penicilânicos. **Química Nova**, v. 33, n. 4, p. 972–977, 2010.

KAUR, S. P.; RAO, R.; NANDA, S. Amoxicillin: a broad spectrum antibiotic. **International Journal of Pharmacy and Pharmaceutical Science**, v. 3, n. 3, p. 30-37, 2011.

NAGARALLI, B. S.; SEETHARAMAPPA, J.; MELWANKI, M. B. Sensitive spectrophotometric methods for the determination of amoxycillin, ciprofloxacin and piroxicam in pure and

pharmaceutical formulations. **Journal of Pharmaceutical and Biomedical Analysis**, v. 29, n. 5, p. 859–864, jul. 2002.

OLIVEIRA, A. F.; SILVA, A. F. S.; TENAN, M. A.; OLIVO, S. L. TitGer: an electronic worksheet for simulating the titration of a mixture of polyprotic species**. Quimica Nova**, v. 30, n. 1, p. 224–228, 2007.

PAN, X.; DENG, C.; ZHANG, D.; WANG, J.; MU, G.; CHEN, Y. Toxic effects of amoxicillin on the photosystem II of Synechocystis sp. characterized by a variety of in vivo chlorophyll fluorescence tests. **Aquatic Toxicology**, v. 89, n. 4, p. 207–213, 2008.

QURESHI, S. Z.; QAYOOM, T.; HELALET, M. I. Simultaneous spectrophotometric and volumetric determinations of amoxycillin, ampicillin and cloxacillin in drug formulations: Reaction mechanism in the base catalysed hydrolysis followed by oxidation with iodate in dilute acid solution. **Journal of Pharmaceutical and Biomedical Analysis**, v. 21, n. 3, p. 473–482, nov. 1999.

RELE, R. V.; MALI, R. N. Simultaneous determination of amoxicillin trihydrate and bromhexine hydrochloride in pharmaceutical dosage by reverse phase high performance liquid chromatography. **Der Pharma Chemica**, v. 5, n. 1, p. 273–278, 2013.

SALEH, G. A. Two Selective Spectrophotometric Methods for the Determination of Amoxicillin and Cefadroxil. **Analyst**, v. 121, may. 1996.

Silva, M. A. M.; Ferreira, M. H.; Braga, J. W. B.; Sena, M. M. Development and analytical validation of a multivariate calibration method for determination of amoxicillin in suspension formulations by near infrared spectroscopy. **Talanta**, v. 89, p. 342–351, jan. 2012.

WALASH, M. I.; TOUBAR, S.; AHMED, S. M.; ZAKHARI, N. A. Spectrophotometric Studies On Determination of Some Cephalosporins and Amoxycillin With Chlorobenzotriazole and Sodium Hypochlorite. **Analytical Letters**, v. 27, n. 13, p. 2499–2513, 1994.