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# Characteristics, Properties and Analytical Methods of Amoxicillin: A Review with Green Approach

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**ABSTRACT**

Bacterial infections are the second leading cause of global mortality. Considering this fact, it is extremely important studying the antimicrobial agents. Amoxicillin is an antimicrobial agent that belongs to the class of penicillins; it has bactericidal activity and is widely used in the Brazilian health system. In literature, some analytical methods are found for the identification and quantification of this penicillin, which are essential for its quality control, which ensures maintaining the product characteristics, therapeutic efficacy and patient's safety. Thus, this study presents a brief literature review on amoxicillin and the analytical methods developed for the analysis of this drug in official and scientific papers. The major analytical methods found were high-performance liquid chromatography (HPLC), ultra-performance liquid chromatography (U-HPLC), capillary electrophoresis and iodometry and diffuse reflectance infrared Fourier transform. It is essential to note that most of the developed methods used toxic and hazardous solvents, which makes necessary industries and researchers choose to develop environmental-friendly techniques to provide enhanced benefits to environment and staff.

**KEYWORDS**

Amoxicillin; analytical methods; antimicrobial; green chemistry; penicillins

## Introduction

Due to the multiplicity of microorganisms, studies related to the development of new antimicrobial agents should be increasingly encouraged, in order to contribute to the treatment of infectious diseases and combating bacterial resistance (Hoefel and Lautert, 2006).

An important type of antibiotic was discovered accidentaly in 1928 by Alexander Fleming. While studying variants of *Staphylococcus*, Fleming observed a zone of inhibition formed around a fungus that was contaminating his cultures. The antibacterial substance was named penicillin, since it was produced by the *Penicillium* fungus (Brunton et al., 2012; Williams and Lemke, 2013).

Penicillins can be classified into several subcategories, which are differentiated by the chemical structures and antimicrobial activities (Brazil, 2016a). Amoxicillin, which belongs to the aminopenicillins subcategory, was synthesized in 1964 by the introduction of a *p*-hydroxyl group in the side chain of ampicillin, which is another aminopenicillin. In this way, the oral absorption was improved and the side effects, such as diarrhea, were minimized, compared to ampicillin (Holten and Onsusko, 2000; Rolinson and Geddes, 2007; Kaur et al., 2011).

Amoxicillin belongs to the RENAME list (Brazilian List of Essential Medicines) and is widely prescribed in clinical practice for the treatment of otitis media and sinusitis. It is also indicated for the treatment of tonsil, throat, larynx, pharynx, bronchi, lungs and skin infections (Miller, 2002; Rolinson and Geddes, 2007; Kaur et al., 2011).

This antimicrobial agent is easily inactivated by $\beta$-lactamases; therefore, it can be used in combination with $\beta$-lactamase inhibitors, such as clavulanic acid (Miller, 2002; Brazil, 2016b).

In Brazil, two tradenames for amoxicillin can be found as reference drugs, Amoxil$^{TM}$ and Amoxil BD$^{TM}$. They are commercialized in the pharmaceutical dosage forms of powder for oral suspension, hard gelatin capsule and coated tablet. For the combination of amoxicillin and clavulanic acid, the commercial names are Clavulin$^{TM}$, Clavulin BD$^{TM}$, Clavulin ES$^{TM}$ and Clavulin IV$^{TM}$, in powder for oral suspension, coated tablet, powder for extemporaneous preparation and lyophilized powder for solution for injection. All these medicines are produced by Glaxosmithkline pharmaceutical company (GSK) (Brasil, 2016c).

In addition to the association with clavulanic acid, amoxicillin is also found associated with sulbactam, as Trifamox IBL$^{TM}$ and Trifamox IBL BR$^{TM}$, in the pharmaceutical dosage forms of powder for solution for injection, coated tablet and powder for extemporaneous preparation, produced by Bagó do Brasil Laboratories (Brazil, 2016d).

Medley Pharmaceutical Industry produces an association of lansoprazole, clarithromycin and amoxicillin trihydrate, named Pyloripac$^{TM}$, as well as an association of lansoprazole, levofloxacin and amoxicillin trihydrate, named Pyloripac Retrat$^{TM}$.

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Both are marketed in the pharmaceutical dosage form of hard gelatin capsule and delayed release coated tablet. The pharmaceutical company Libbs produces an association of omeprazole, clarithromycin and amoxicillin, named Erradic U.G.$^{TM}$, in the pharmaceutical forms of hard gelatin capsule and coated tablet (Brazil, 2016d).

Due to high demand about the use of amoxicillin worldwide, studies involving its quality control are highly encouraged in order to ensure the standards of reliability and effectiveness of this drug, minimizing the risks for the population. In this context, the development of reliable analytical methods is increasingly recognized and required (La Roca et al., 2007). In addition, antimicrobials with lack of quality contribute to the emergence of the serious problem of bacterial resistance.

Considering the importance of amoxicillin for the clinical practice, this paper aims to review the chemical, pharmaceutical and clinical aspects of this antimicrobial, as well as reviewing the analytical methods described in literature for its analysis. The research was carried out using MEDLINE, Science Direct and Scifinder databases, from January 1950 to June 2016, using English language restriction. The main terms used were: 'amoxicillin' in combination with 'HPLC', 'U-HPLC', 'UPLC', 'spectrometry', 'spectrophotometry', 'agar diffusion', 'analytical methods', 'quantitative analysis', 'microbiological assay', among others. The studies included in this review met the following criteria: original data; data regarding the use of amoxicillin and data regarding analytical methods.

## Amoxicillin

### Structural forms

Amoxicillin is available in three different forms: anhydrous, sodium and trihydrate (Argentina Pharmacopoeia, 2003; BP, 2012; Brazilian Pharmacopoeia, 2010; EP, 2013; IP, 2007; JP, 2011; USP 37, 2014). Figure 1 illustrates the chemical structures.

### Mechanism of action

All $\beta$-lactam antibiotics are bactericidal agents that interfere with the biosynthesis of peptidoglycan, one of the constituents of the bacterial cell wall. This structure is necessary for the maintenance of cell integrity, among other vital processes (Miller, 2002; Kaur et al., 2011). The peptidoglycan is composed of two alternate amino sugars (N-acetylglucosamine and N-acetylmuramic acid) and a peptide of five amino acids ending in D-Alanyl-D-Alanine, which is attached to the N-acetylmuramic acid. This polymer provides rigid mechanical stability due to its highly cross-linked structure (Petri, 2006).

Amoxicillin binds to the penicillin binding protein 1A (PBP-1A), which is located inside the bacterial cell wall, and inactivates the penicillin-sensitive transpeptidase by acylation of its C-terminal domain. This interaction is possible because amoxicillin has a structure similar to the D-Alanyl-D-Alanine, the original substrate for this enzyme. This enzyme inhibition occurs in the last step of the synthesis of the bacterial cell wall, thus preventing cross-linking of two linear peptidoglycan chains (Petri, 2006). Structural destabilization of the cell wall leads to the formation of spheroplasts and rapid occurrence of lysis mediated autolytic enzymes (Petri, 2006; Kaur et al., 2011).

### Microbiological spectrum and clinical use

Amoxicillin is active against *Streptococcus pyogenes* and many strains of *Streptococcus pneumoniae* and *Haemophilus influenzae*, which are causative microorganisms of bacterial infections of the upper respiratory tract. For this reason, it is indicated in cases of tonsil, throat, larynx and pharynx infections and may also act on the bronchi and lungs (Petri, 2006; Kaur et al., 2011). Amoxicillin is the most active of all oral $\beta$-lactam antibiotics against *S. pneumoniae*, and it is first choice drug for the treatment of maxillary sinusitis and otitis media (Miller, 2002).

This penicillin may also be used for the treatment of uncomplicated urinary tract infections caused by enterobacteria such as *Escherichia coli*. Amoxicillin is also used as prophylaxis against bacterial endocarditis and before genitourinary and gastrointestinal procedures (Miller, 2002; Petri, 2006; Rolinson, Geddes 2007; Kaur et al., 2011).

### Pharmacokinetics and pharmacodynamics

Amoxicillin has high oral bioavailability (70–90%), which is not interfered by the presence of food in the gastrointestinal tract. The plasma concentration peaks occur after 1–2 hours, depending on the dose. About 20% of amoxicillin is bound to plasma proteins (Williams and Lemke, 2013).

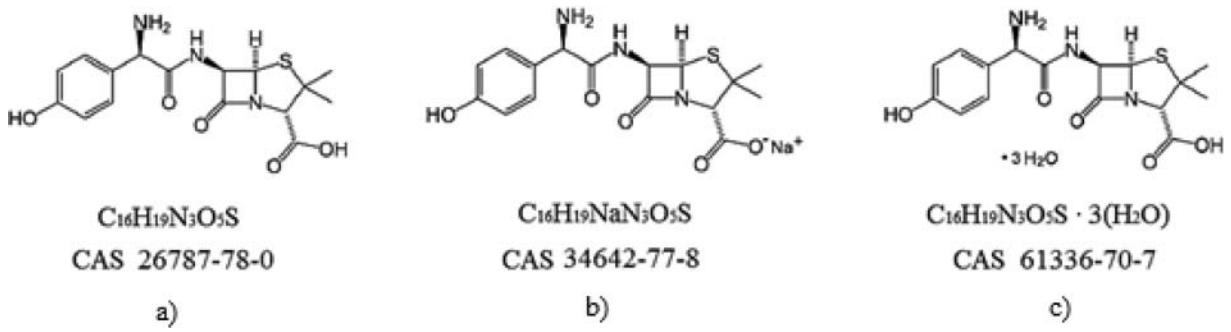


**Figure 1.** Chemical structures of amoxicillin anhydrous (a), amoxicillin sodium (b) and amoxicillin trihydrate (c).

**Table 1.** Analytical methods described in literature for determination of amoxicillin in pharmaceutical dosage forms and raw material.

| Method | Conditions | Detection system | Matrix | Reference |
|---|---|---|---|---|
| PIXE | Amoxicillin was analyzed in their tablets 'as received' form, in pellets made from the powder of the tablets and also in pellets made from the powder of the tablets after being heated up to 70°C. | Not applicable | Tablets | Bejjani et al. (2016) |
| Colorimetry | The system was based on aggregation of citrate-capped gold nanoparticles in acetate buffer (pH = 4.5) in the presence of the degradation product of amoxicillin (DPAMX). The absorbance ratio at the wavelength of 660 and 525 nm ($A_{660}$/$A_{525}$) was chosen as the analytical signal indirectly related to amoxicillin concentration. | UV/Vis (600–700 nm) | Capsules | Akhond et al. (2015) |
| HPLC | Thermosil $C_{18}$ analytical column (250 × 4.6 mm; 5$\mu$m). Mobile phase: potassium dihydrogen phosphate buffer (adjusted to pH 3 by ortho phosphoric acid) and methanol (70:30, v/v). Flow rate: 1.0 mL/min. Rt: 2.582 min. | UV/DAD (225 nm) | Bulk and capsules | Konari and Jacob (2015) |
| U-HPLC | Shim-Pack XR-ODS analytical column (50 × 2.0 mm; 2.2 $\mu$m). Mobile phase: methanol and 0.0025 M ammonium acetate, in the initial ratio 40:60 (v/v) at a gradient program. Flow rate: 0.25 mL/min. Rt: 0.75 min. | UV (220 nm) | Tablets and capsules | Uddin et al. (2015) |
| HPLC | LC- 20 AT $C_{18}$ column (250 × 4.6 mm; 2.6$\mu$m) at 30°C. Mobile phase: buffer with pH 4.5 and acetonitrile (40:60, v/v). Flow rate: 1.0 mL/min. Rt: 9.0 min. | UV (277 nm) | Raw material | Vaidya et al. (2015) |
| HPLC | Shim-pack VP-ODS column (250 × 4.6 mm). Mobile phase: phosphate buffer pH 4.4 and methanol (91:9, v/v). Flow rate: 2.0 mL/min. Rt: 4.9 min. | UV (220 nm) | Tablets | Muchlisyam and Alfan (2014) |
| Capillary electrophoresis | Polyimidecoated fused silica-capillaries of 56 cm (effective length: 48 cm) × 50 $\mu$m I.D. (Agilent). Electrolyte: 25 mM sodium tetraborate buffer and 100 mM sodium dodecyl sulphate, pH 9.3, as surfactant. Voltage: +25 kV. Temperature: 25°C. Migration time: 3.41 min. | UV (210 nm) | Raw material | Simon et al. (2014) |
| Iodometry | Potassium monopersulfate was used as reagent. | Not applicable | Raw material | Blazhevskii et al. (2013) |
| HPLC | $C_{18}$ ACE column (150 × 4.6 mm; 3 $\mu$m). Mobile phase: phosphoric acid solution and acetonitrile (90:10 v/v). Flow rate: 1.0 mL/min. Rt: 10.0 min. | UV (220 nm) | Veterinary medicines | Miguel et al. (2013) |
| HPLC | $C_{18}$ Hypersil ODS column (250 × 4.6 mm). Mobile phase: acetate buffer pH 4.0 and methanol (94:6 v/v). Flow rate: 1.0 mL/min. Rt: 7.03 min. | UV (228 nm) | Injectable | Rajesh et al. (2013) |
| HPLC | $C_{18}$ column. Mobile phase: 0.1 M monopotassium phosphate, 0.018 M sodium dodecyl sulfate (SDS), methanol and acetonitrile (275: 275: 200: 250, v/v/v/v), pH adjusted to 2.6 with phosphoric acid. | UV (225 nm) | Injectable suspension | Xue and Liao (2013) |
| HPLC | $C_{18}$ column (250 × 4.6 mm; 5 $\mu$m). Mobile phase: acetonitrile and water (10:90, v/v), pH adjusted to 3.0 with orthophosphoric acid. Flow rate: 1.0 mL/min. Rt: 5.1 min. | UV (220 nm) | Pharmaceutical dosage forms | Vispute et al. (2013) |
| HPLC | YILITE $C_{18}$ column (150 × 4.6 mm; 5 $\mu$m). Mobile phase: phosphate buffer solution (pH adjusted to 4.4 ± 0.1 with 10 M sodium hydroxide) and methanol (95:5, v/v). Flow rate: 1.0 mL/min. | UV (220 nm) | Oral suspension | Zhang et al. (2013) |
| HPLC | MG Capacel Pak $C_{18}$ column. Mobile phase: phosphate buffer solution and methanol (50:50, v/v), pH 3.0. Flow rate: 1.0 mL/min. | UV (229 nm) | Bulk and pharmaceutical dosage forms | Beg et al. (2012) |
| HPLC | Phenomenex Luna $C_{18}$ (2) column (250 × 4.6 mm; 5 $\mu$m). Mobile Phase: 25 mM/L potassium dihydrogen phosphate, (pH adjusted to 4.5 with orthophosphoric acid) and acetonitrile (40:60, v / v). Flow rate: 1.0 mL/min. Rt: 2.053 min. | UV (238 nm) | Tablets | Bojaraju et al. (2012) |
| HPLC | Dikma Diamonsil $C_{18}$ column (250 × 4.6 mm; 10 $\mu$m), at 40°C. Mobile phase: phosphoric acid buffer solution (pH 5.0) and acetonitrile (98:2 v/v) | UV (254 nm) | Oral suspension | Lu et al. (2012) |
| HPLC | Symmetry XTERRA $C_{18}$ column (150 × 4.6 mm; 5 $\mu$m). Mobile phase: phosphate buffer solution (pH 2.5) and acetonitrile (40:60, v/v). Flow rate: 0.8 mL/min. Rt: 4.1 min. | UV (228 nm) | Tablets | Nadiminti et al. (2012) |
| HPLC | Inertsil ODS-2 column (150 × 4.6 mm; 5 $\mu$m), at 25°C. Mobile phase: methanol and 0.01 M ammonium acetate (52:48, v/v). Flow rate: 1.0 mL/min. | UV (247 nm) | Dry suspensions | Song and Chen (2012) |
| HPLC | Kromasil $C_{18}$ column. Mobile phase: 0.05 M monosodium phosphate and methanol (95:5, v/v). Flow rate: 1.0 mL/min. | UV (220 nm) | Injectable | Zou et al. (2012) |
| HPLC | Capcell Pak $C_{18}$ column. Mobile phase: methanol and 0.02 M phosphate buffer solution (50:50, v/v, pH 3.5). Flow rate: 1.0 mL/min. Rt: 3.99 min. | UV (229 nm) | Tablets | Beg et al., 2011 |
| HPLC | Agilent Zorbax Eclipse Plus $C_{18}$ column (150 × 2.1 mm; 3.5 $\mu$m) at 35°C. Mobile phase: solution A (water and 0.1% formic acid) and solution B (acetonitrile and 0.1% formic acid) (90:10, v/v). Flow rate: 9.0–11.0 mL/min. | MS | Raw material | Dinha et al. (2011) |

(*Continued on next page*)

**Table 1.** (*Continued*).

| Method | Conditions | Detection system | Matrix | Reference |
|---|---|---|---|---|
| HPLC | $C_{18}$ column. Mobile phase: solution A (0.02 M monopotassium phosphate, pH adjusted to 6.5 with 2 M NaOH containing 1.5% cetyltrimethylammonium bromide, and acetonitrile [60:40, v/v]) and solution B (0.02 M monopotassium phosphate, pH adjusted to 6.5 with the same solution of NaOH and acetonitrile [30:70, v/v]) | UV (254 nm) | Granules | Lu and Wang (2011) |
| HPLC | $C_{18}$ Hypersil column (250 × 4.6 mm; 5$\mu$m). Mobile phase: potassium phosphate monobasic and methanol (95:05, v/v). Flow rate: 1.0 mL/min. Rt: 6.30 min. | UV (283 nm) | Pharmaceutical preparation | Manzoor et al. (2011) |
| HPLC | $C_{18}$ column HiQ Sil (250 × 4.6 mm; 5$\mu$m). Mobile phase: methanol and 0.02 M ammonium acetate (90:10, v/v) with pH 5.0 adjusted with 10% orthophosphoric acid. Flow rate: 1.0 mL/min. | UV (254 nm) | Capsules | Dhoka et al. (2010) |
| HPLC | $C_{18}$ Apollo column (150 × 4.6 mm; 5$\mu$m) Mobile phase: 0.01 M potassium phosphate monobasic and methanol (45:55, v/v). Flow rate: 1.0 mL/min. Rt: 1.57 min. | UV/DAD (225 nm) | Capsules | Giang and Honag (2010) |
| HPLC | $C_{18}$ Hypersil column (250 × 4.6 mm; 5$\mu$m). Mobile phase: phosphate buffer and methanol (95:05, v/v). Flow rate: 1.0 mL/min. | UV (220 nm) | Capsules | Jayakar et al. (2010) |
| HPLC | VertiSep™ GES ODS column (150 × 4.5 mm; 5$\mu$m). Mobile phase: monobasic potassium phosphate solution, pH 5.0 and acetonitrile (96:4, v/v). Flow rate: 0.8 mL/min. | UV/DAD (230 nm) | Raw material | Rojanarata et al. (2010) |
| HPLC | $C_{18}$ column (250 × 4.5 mm; 5 $\mu$m). Mobile phase: methanol and glacial acetic acid (50:50, v/v). Flow rate: 1.0 mL/min. Rt: 3.04 min. | UV (254 nm) | Oily suspension | Sonawane and Bari (2010) |
| HPLC | $C_{18}$ Inertsil column (250 × 4.0 mm; 4 $\mu$m). Mobile phase: monopotassium phosphate with pH adjusted to 5.0 with orthophosphoric acid, and methanol (95:5, v/v). Flow rate: 1.0 mL/min. Rt: 7.5 min. | UV (220 nm) | Injectable | Tippa and Singh (2010) |
| HPLC | Spherisorb ODS-2 column (250 × 4 mm; 5$\mu$m) at 40°C. Mobile phase: phosphate buffer with pH 3.5 and acetonitrile (35:65, v/v). Rt: 4.0 min. | UV (355 nm) | Raw material | Delis et al. (2009) |
| HPLC | $C_{18}$ Kromasil column (250 × 4.6 mm; 5 $\mu$m). Mobile phase: 0.02 M dihydrogen potassium orthophosphate and acetonitrile (75:25, v/v). Flow rate: 1.5 mL/min. | UV (225 nm) | Capsules | Nikam et al. (2009) |
| HPLC | Luna C18 column (150 × 4.6 mm; 5$\mu$m) at 50°C. Mobile phase: acetonitrile and water (25:75, v/v). Flow rate: 1.5 mL/min. Rt: 5.0 min. | UV (220 nm) | Standard | Peña et al. (2009) |
| HPLC | $C_{18}$ Kromasil column (150 × 4.6 mm; 5 $\mu$m). Mobile phase: acetonitrile and 0.02 M potassium phosphate monobasic. Flow rate: 1.0 mL/min. | UV (230 nm) | Capsules | Qi et al. (2009) |
| HPLC | $C_{18}$ YMG column (250 × 4.6 mm; 10 $\mu$m). Mobile phase: 0.015 M sodium dodecyl sulfate and 0.03 M sodium acetate (pH adjusted to 4.2 with acetic acid) and methanol (80:20, v/v). Flow rate: 1.0 mL/min. | UV (225 nm) | Capsules | Qin et al. (2009) |
| HPLC | $C_{18}$ column. Mobile phase: 0.05 M potassium phosphate monobasic and acetonitrile (97.5:2.5, v/v). Flow rate: 1.0 mL/min. | UV (230 nm) | Granules | Hong et al. (2009) |
| HPLC | Shim-Pack VP-ODS column (150 × 4.6 mm; 5 $\mu$m). Mobile phase: phosphate buffer pH 5.0 and ethanol (90:10, v/v). Flow rate: 1.0 mL/min. | UV (228 nm) | Capsules | Li et al. (2008) |
| DRIFTS | The analysis was conducted in the mid-infrared region. Data were analyzed using regression by partial least squares. | Not applicable | Mixture of amoxicillin and starch | Parisotto et al. (2007) |
| HPLC | Whatman Par tisil 5 ODS-3 column (100 × 4.6 mm; 5 $\mu$m). Mobile phase: 0.05 M phosphate buffer pH 4.0 and methanol (95:5, v/v). Flow rate: 1.5 mL/min. Rt: 3.2 min. | UV (254 nm) | Tablets | Tavakoli et al. (2007) |
| HPLC | $C_{18}$ Phenomenex column (150 × 4.6 mm; 5 $\mu$m) at 30°C. Mobile phase: 0.78% monosodium phosphate pH 4.4 and methanol (95:5, v/v). Flow rate: 0.8 mL/min. | UV (220 nm) | Granules | Zhang (2007) |
| HPLC | Shimadzu VP-ODS column (250 × 4.6 mm). Mobile phase: 0.008 M sodium dodecyl sulfate (pH adjusted to 4.5 with phosphoric acid) and methanol (68:32, v/v). Flow rate: 1.0 mL/min. | UV (225 nm) | Capsules | Hong et al. (2006) |
| HPLC | Gel filtration Ohpak SB-802.5 HQ column. Mobile phase: phosphate buffer solution. Flow rate: 0.8 mL/min. | Not available | Capsules and suspensions | Li et al. (2006) |
| HPLC | $C_{18}$ Nucleosil 120 column (250 × 4.6 mm; 10 $\mu$m). Mobile phase: solution A (methanol) and solution B (buffer solution pH 3.0). Gradient elution. Flow rate: 1.75 mL/min. | UV/DAD (230 nm) | Veterinary formulation (Premix™) | Pérez-Lozano et al. (2006) |

(*Continued on next page*)

**Table 1.** (*Continued*).

| Method | Conditions | Detection system | Matrix | Reference |
|---|---|---|---|---|
| HPLC | $C_{18}$ Agilent Zorbax SB column (150 × 4.6 mm; 5 $\mu$m). Mobile phase: water, methanol, phosphoric acid and triethylamine (842:150:4:4, v/v/v/v), containing hexane-1-sulfonic acid, 10 mM sodium salt (pH adjusted to 3.5 with phosphoric acid). Flow rate: 0.8 mL/min. | UV (230 nm) | Veterinary formulation (Premix$^{TM}$) | Dousa and Hosmanova (2005) |
| HPLC | ZORBAX 300-SCX column (250 × 4.6 mm; 5 $\mu$m). Mobile phase: ammonium dihydrogen phosphate (pH adjusted to 2.6 with phosphoric acid) and acetonitrile (95:5, v/v). Flow rate: 1.5 mL/min. | UV (225 nm) | Injectable | Liu et al. (2005) |
| HPLC | Zorbax RX $C_8$ column (150 × 2.1 mm; 5 $\mu$m). Mobile phase: formic acid, water and acetonirile (2:1000:100, v/v/v). Flow rate: 0.4 mL/min. | MS | Human plasma | Yoon et al. (2004) |
| HPLC | $C_{18}$ YWG column (250 × 4.6 mm; 5 $\mu$m). Mobile phase: 0.01 M sodium dodecyl sulfate (pH adjusted to 4.2 with phosphoric acid) and methanol (60:40, v/v). Flow rate: 1.0 mL/min. | UV (225 nm) | Capsules | Yin et al. (2005) |
| HPLC | $C_{18}$ column. Mobile phase: solution A (0.05 M phosphate buffer pH 5.0 and acetonitrile [99:1, v/v]) and solution B (0.05 M phosphate buffer solution pH 5.0) (80:20, v/v). Gradient elution. | UV (254 nm) | Pharmaceutical preparations | Zhou et al. (2005) |
| HPLC | Hypersil $C_{18}$ column (250 × 4.6 mm; 10 $\mu$m). Mobile phase: methanol and phosphate buffer pH 5.0 (52:48, v/v). Flow rate: 1.0 mL/min. | UV (230 nm) | Tablets | Chen and Zhang (2003) |
| HPLC | Lichrosphere $C_{18}$ column. Mobile phase: methanol, acetonitrile and phosphate buffer. | UV (210 nm) | Mixture of five penicillins | Kaniou et al. (1993) |
| HPLC | $C_{18}$ $\mu$Bondapak column (300 × 3.9 mm; 10 $\mu$m). Mobile phase: 1.25% methanol and acetic acid (20:80, v/v). Flow rate: 1.5 mL/min. Rt: 3.8 min. | UV (254 nm) | Pharmaceutical preparations | Hsu and Hsu (1992) |
| HPLC | $C_{18}$ Chromegabond column. Mobile phase: acetonitrile, methanol and 1 M potassium phosphate buffer pH 4.7 (19:11:70, v/v/v). | UV (225 nm) | Mixture of nine penicillins | Briguglio and Lau-Cam (1984) |

PIXE: Particle Induced X-ray Emission; HPLC: High performance liquid chromatography; U-HPLC: Ultra-high performance liquid chromatography; UV: ultraviolet detector; DAD: diode-array detector; DRIFTS: Diffuse reflectance infrared Fourier transform; MS: Mass spectrometry.

When orally administered, it keeps stable in acid medium; it is well absorbed by gastrointestinal tract and shows good penetration into tissues, such as the liver, lungs, prostate gland, muscle and synovial, pleural and amniotic fluids, being able to cross the placenta. Inflammations generally cause an increase of meningeal permeability to penicillins. Amoxicilin has a half-life of elimination of approximately 1.0–1.5 hours, and the kidney is its main route of elimination (Katzung, 2010; Kaur et al., 2011; Brazil, 2016b).

Concomitant use of probenecid and amoxicillin causes delays in the excretion of the penicillin. About 10–25% of the drug is eliminated as inactivated penicilloic acid, while the remainder is excreted in its unchanged form (Kaur et al., 2011).

### *Physicochemical properties*

Amoxicillin is a semi-synthetic antibiotic belonging to the class of penicillins and subclass of aminopenicillins. Its hydrate form is used for the pharmaceutical dosage form of capsule. The hydrate form is chemically known as (2S,5R,6R)-6-[(2R)-2-amino-2-(4-hydroxyphenyl)-acetylamino]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid trihydrate (The Merck Index, 2006; USP 37, 2014). Its molecular formula is $C_{16}H_{19}N_3O_5S$, with molecular mass of 365.404 g/mol (Brazilian Pharmacopoeia, 2010).

Amoxicillin has poor solubility in water, ethanol and methanol; it is insoluble in benzene, ethyl acetate, chloroform, diethyl ether and acetonitrile, and presents high solubility in hydroxide alkali solution. Its melting point is 194°C and its dissociation constants (pKa) are 2.4 (carboxylic acid), 7.4 (amine) and 9.6 (phenol) (Brazilian Pharmacopoeia, 2010; Rolinson and Geddes, 2007; The Merck Index, 2006; USP 37, 2014).

## Analytical methods for the analysis of amoxicillin

The development of analytical methods for the analysis of amoxicillin in different matrixes is very relevant, mainly to assist bioavailability, bioequivalence and pharmacokinetic studies, as well as monitoring the quality of the marketed product.

There are several studies described in literature regarding the development of analytical methods for qualitative and quantitative analysis of amoxicillin using high-performance liquid chromatography (HPLC) (in most cases), ultra-performance liquid chromatography (U-HPLC), iodometry, diffuse reflectance infrared Fourier Transform spectroscopy (DRIFTS), capillary electrophoresis, particle induced X-ray emission (PIXE), colorimetry, among others. These methods aim to analyze amoxicillin in different matrixes, such as pharmaceutical dosage forms, raw material, biological fluids, food of animal origin, among others.

Table 1 presents the analytical methods found in literature for the analysis of amoxicillin in pharmaceutical dosage forms and raw material. Table 2 shows the analytical methods found to determine amoxicillin in biological fluids, food of animal origin, and other matrixes. Table 3 presents the methods suggested by pharmacopoeial compendia.

Regarding the analytical methods described in literature for the analysis of amoxicillin in different matrixes, it is

**Table 2.** Analytical methods described in literature for determination of amoxicillin in biological fluids, food of animal origin and other matrixes.

| Method | Conditions | Detection system | Matrix | Reference |
|---|---|---|---|---|
| U-HPLC | Acquity UPLC™ BEH RP $C_{18}$ analytical column (50 × 2.1 mm; 1.7$\mu$m). Mobile phase: solvent A (0.1% formic acid in water) and solvent B (0.1% formic acid in acetonitrile), under a gradient program. Flow rate: 0.3 mL/min. Rt: 1.87 min. | MS | Cows' milk | Liu et al. (2016) |
| HPLC | Agilent $C_{18}$ analytical column (50 × 4.6 mm; 5$\mu$m) protected with a guard column ($C_{18}$; particle size 5$\mu$m). Mobile phase: solvent A (acetonitrile) and solvent B (0.1% formic acid in water), under a gradient program. Flow rate: 1.0 mL/min. Rt: 4.99 min. | MS | Eggs | Sun et al. (2016) |
| HPLC | $C_{18}$ endcapped column (70 × 4.6 mm; 5 $\mu$m), coupled to a $C_{18}$ pre-column (4.0 × 4.6 mm), at 25 °C. Mobile phase: solution A (50 mM phosphate buffer pH 3.0) and solution B (methanol). Flow rate: 1.0 mL/min and gradient elution. Rt: 1.69 min. | UV/DAD (230 nm) | Residual water | Vosough et al. (2015) |
| HPLC | Thermo Hypersil BDS-$C_8$ analytical column (250 × 4.6 mm; 5 $\mu$m). Mobile phase: phosphate buffer pH 7.0 and acetonitrile (70: 30, v/v). Flow rate: 1.0 mL/min. Rt: 2.36 min. | UV/DAD (230 nm) | Simulated intestinal fluid | Sabry et al. (2015) |
| U-HPLC | Acquity UPLC BEH $C_{18}$ column (100 × 2.1 mm). Mobile phase: metanol and 0.1% formic acid solution. Flow rate: 0.3 mL/min. Rt: 6.0 min. | MS | Human urine, serum, cerebrospinal fluid and bronchial aspiration | Carzola et al. (2014) |
| HPLC | $C_{18}$ ODS-BP column (250 × 4.6 mm; 5 $\mu$m) coupled to a $C_{18}$ pre-column, at 30°C. Mobile phase: 0.1 M acetonitrile solution and phosphate buffer solution pH 6.5 (25:75, v/v), containing 0.015 M tetrabutylammonium bromide and sodium thiosulfate. Flow rate: 1.0 mL/min. | UV (325 nm) | Eggs | Ma et al. (2014) |
| HPLC | Acquity HSS $T_3$ column (50 × 2.1 mm; 1.7 $\mu$m). Mobile phase: solution A (acetic acid and amonium acetate buffer) and solution B (acetonitrile). Flow rate: 0.6 mL/min. Rt: 5.0 min. | MS | Human plasma | Collin et al. (2013) |
| HPLC | HyPURITY advance $C_{18}$ column (50 × 4.6 mm; 5 $\mu$m). Mobile phase: ammonium acetate and acetonitrile (20:80, v/v). Flow rate: 1.5 mL/min. | MS | Human plasma | Gaikwad et al. (2013) |
| HPLC | $C_{18}$ ACE column (150 × 4.6 mm; 3 $\mu$m). Mobile phase: acetonitrile and water. Flow rate: 0.4 mL/min. Rt: 9 min. | MS | Human blood | Szultka et al. (2013a) |
| HPLC | $C_8$ ACE column (150 × 4.6 mm; 5 $\mu$m). Mobile phase: methanol and water (50:50, v/v). Flow rate: 0.45 mL/min. Rt: 7 min. | MS | Human plasma | Szultka et al. (2013b) |
| HPLC | $C_{18}$ Athena column (250 × 4.6 mm; 5 $\mu$m). Mobile phase: solution A (acetonitrile) and solution B (0.01 M dihydrogen phosphate). Flow rate: 1.0 mL/min. | Fluorimetry | Albumine | Xie et al. (2013) |
| U-HPLC | $C_{18}$ column (100 × 2.1 mm; 1.7$\mu$m). Mobile phase: formic acid in water and formic acid in acetonitrile. Gradient elution. Flow rate: 0.4 mL/min. Rt: 5 min. | MS | Human plasma | Carlier et al. (2012) |
| U-HPLC | Acquity UPLC BEH Waters $C_{18}$ column (50 × 2.1 mm; 1.7 $\mu$m) at 30°C. Mobile phase: 0.1% formic acid in water and 0.1% formic acid in acetonitrile (50:50, v/v). Flow rate: 0.3 mL/min. | MS | Bovine milk | Li et al. (2012) |
| HPLC | Mobile phase: 0.01 M monopotassium phosphate and acetonitrile. Flow rate: 1.0 mL/min. | Fluorimetry | Eggs | Xie et al. (2012) |
| HPLC | $C_{18}$ column. Mobile phase: solution A (methanol) and solution B (0.1% formic acid). Gradient elution. | MS | Honey | Xu et al. (2012) |
| HPLC | $C_{18}$ HyPURITY column (150 × 2.1 mm; 3.5 $\mu$m). Mobile phase: 0.1% formic acid and acetonitrile. | MS | Fish derivatives | Guo et al. (2011) |
| HPLC | Waters Acquity UPLC HSS $T_3$ column (100 × 2.1 mm; 1.8 $\mu$m) at 30°C. Mobile phase: acetonitrile, 0.15% formic acid in water with 5 mM ammonium acetate. Flow rate: 0.25 mL/min. | MS | Bovine milk | Liu et al. (2011) |
| HPLC | Waters Atlantis $T_3$ column (150 × 2.1 mm; 3 $\mu$m). Mobile phase: solution A (acetonitrile) and solution B (water with 0.005% formic acid). Gradient elution. Flow rate: 0.2 mL/min. Rt: 8.0 min. | MS | Bovine muscle | Lugoboni et al. (2011) |
| HPLC | Inertsil ODS column (150 × 4.6 mm; 5 $\mu$m) at 30°C. Mobile phase: mixture of 30 mM potassium dihydrogen phosphate buffer (pH 2.8) and acetonitrile (97.5:2.5, v/v). Flow rate: 1.0 mL/min. Rt: 6.0 min. | UV/DAD (210 nm) | Human plasma | Pei et al. (2011) |
| HPLC | Atlantis $T_3$ column (150 × 4.6 mm; 5 $\mu$m) coupled to a pre-column (20 × 4.6 mm; 5 $\mu$m). Mobile phase: solution A (10 mM phosphoric acid solution, adjusted to pH 2.0 with HCl) and solution B (acetonitrile). Flow rate: 2.0 mL/min and gradient elution. Rt: 3.4 min. | UV (230 nm) | Human plasma | Verdier et al. (2011) |
| HPLC | $C_{18}$ column (250 mm × 4.6 nm; 5 $\mu$m). Mobile phase: potassium phosphate monobasic buffer and acetonitrile (96:4 v/v). Flow rate: 1.5 mL/min. | UV (230 nm) | Cleaning waste | Gomes and Souza (2010) |

(*Continued on next page*)

**Table 2.** (*Continued*).

| Method | Conditions | Detection system | Matrix | Reference |
|---|---|---|---|---|
| HPLC | Phenomenex Hydro $C_{12}$ column (50 × 2.0 mm). Mobile phase: water and methanol. Flow rate: 0.7 mL/min. Rt: 3.0 min. | *MS* | Bovine feed | Kantiani et al. (2010) |
| HPLC | Phenomenex Gemini $C_{18}$ column (150 × 4.6 mm; 5 $\mu$m) at 30°C. Mobile phase: formic acid 0.1% in water, methanol and acetonitrile (94:3:3, v/v/v). Flow rate: 1.0 mL/min. Rt: 6.76 min. | UV/DAD (230 nm) | Human urine | Torres et al. (2010) |
| HPLC | Chromolith Performance $C_{18}$ endcapped column (100 × 4.6 mm). Mobile phase: solution A (0.1% trifluoroacetic acid) and solution B (100% acetonitrile and 0.1% trifluoroacetic acid. Gradient elution. Flow rate: 3.0 mL/min. | UV (210 nm) | Animal feed | Injac et al. (2009) |
| HPLC | Polymer PLRP-S column (150 × 2.1 mm) coupled to pre-column (10 × 2.0 mm), at 25°C. Mobile phase: solution A (0.1% formic acid) and solution B (acetonitrile). Gradient elution. Flow rate: 0.2 mL/min. Rt: 3.1 min. | MS | Swine tissue | Reyns et al. (2008) |
| HPLC | Chromolith Performance $C_{18}$ column (100 × 4.6 mm). Mobile phase: mixture of 0.02 M disodium hydrogen phosphate buffer and methanol (4:96, v/v) adjusted to pH 3.0. Flow rate: 1.3 mL/min. Rt: 3.8 min. | UV (228 nm) | Human plasma | Foroutan et al. (2007) |
| HPLC | Zorbax SB $C_{18}$ column (150 × 4.6 mm) at 25°C. Mobile phase: phosphate buffer with pH 5.0 and acetonitrile (95:5, v/v). Flow rate: 1.0 mL/min. Rt: 2.5 min. | UV (230 nm) | Urine | Reiriz et al. (2007) |
| Capillary electrophoresis | Fused-silica capillary of 58.5 cm (effective length: 75$\mu$m). Electrolyte: 2.7 × $10^{-2}$ M potassium dihydrogenphosphate and 4.3 × $10^{-2}$ M sodium tetraborate solution, with pH 8. Voltage: +18 kV. Temperature: 25°C. | UV (210 nm) | Milk | Santos et al. (2007) |
| HPLC | $C_{18}$ Symmetry column (150 × 4.6 mm; 5 $\mu$m). Mobile phase: metanol and 75 mM phosphate buffer with pH adjusted to 3.0 with phosphoric acid (10:90, v/v). Flow rate: 1.5 mL/min. Rt: 6.9 min. | UV (228 nm) | Human plasma | Matar (2006) |
| HPLC | Phenomenex $C_{18}$ column (250 × 4.6 mm; 10$\mu$m) with a$\mu$Bondpak $C_{18}$ Guard-pak precolum. Mobile phase: phosphate buffer (pH 3.0; 0.023 M) containing 4 mM 1-octanesulphonic acid sodium and acetonitrile (87:13, v/v). Flow rate: 1.0 mL/min. Rt: 10.0 min. | UV (210 nm) | Mouse serum and broncho-alveolar lavage fluid | Du et al. (2005) |
| HPLC | Zorbax RX $C_8$ column (150 × 2.1 mm; 5 $\mu$m). Mobile phase: formic acid, water and acetonirile (2:1000:100, v/v/v). Flow rate: 0.4 mL/min. | MS | Human plasma | Yoon et al. (2004) |
| HPLC | LiChrospher $C_{18}$ column (125 × 4.0 mm; 5 $\mu$m) at 35°C. Mobile phase: methanol and phosphate buffer pH 7.2 (7.5:92.5, v/v). Flow rate: 1.5 mL/min. Rt: 10.0 min. | UV (225 nm) | Plasma | Leroy et al. (1992) |

HPLC: High performance liquid chromatography; U-HPLC: Ultra-high performance liquid chromatography; UV: ultraviolet detector; DAD: diode-array detector; MS: Mass spectrometry

possible to observe that HPLC is the most used method. However, this technique has some disadvantages that need to be mentioned. It requires expensive equipment and analytical columns, makes use of large volumes of solvents as mobile phase, and it is a method of high maintenance cost. In addition, this method can be harmful to the environment, since most papers found in literature describe the use of toxic organic solvents, such as methanol and acetonitrile, as mobile phases. In these studies, the mobile phases were adjusted to pH between 2.5 and 6.5, and the retention times ranged from 2.053 to 10.0 minutes. Some papers describe the use of pH-correcting agents, such as formic acid, phosphoric acid and trifluoroacetic acid.

On the other hand, environmentally friendly analytical methods have been identified, such as capillary electrophoresis (Santos et al., 2007; Simon et al., 2014), spectroscopy by diffuse reflectance infrared Fourier transform (Parisotto et al., 2007) and U-HPLC (Li et al., 2012; Carlier et al., 2012; Carzola et al., 2014; Uddin et al., 2015; Liu et al., 2016). Furthermore, Li et al. (2008) describe a green HPLC method for determination of amoxicillin in capsules using ethanol as organic phase of the mobile phase, and this solvent is less harmful to operator's health and the environment. However, the suggested mobile phase has phosphate buffer solution (pH 5.0). The use of buffer solution can damage the chromatographic system and the chromatographic column due to precipitation of salts. After using buffer solutions, the operators have to perform long washes in the chromatographic system in order to avoid such problems, which leads to greater waste of time, energy and water for cleaning (Santos-Neto, 2009, 2010; Tótoli and Salgado, 2014, 2015; Rodrigues and Salgado, 2016).

The detection system adopted by most HPLC methods was ultraviolet, in the wavelength ranged between 210 and 325 nm. Fluorometry was also used, as well as diode array detector (DAD) and mass spectrometry. The used stationary phases are commonly $C_{18}$ analytical columns.

The choice of reliable analytical methods for the analysis of medicines is extremely important. These methods must be continuously optimized, aiming to improve patient safety, reduce production costs and electric energy consumption, and minimize the impacts on the environment and health risks to operators. Green Chemistry is highly relevant nowadays, since it contributes directly to the environmental and economic impacts, sustainable social production and good management practices (Anastas, 2016; Hodges, 2016; Nies, 2016).

**Table 3.** Analytical methods described in pharmacopoeial compendia for determination of amoxicillin.

| Method | Conditions | Detection system | Matrix | Reference |
|---|---|---|---|---|
| HPLC | L1 column (250 × 4.0 mm). Mobile phase: solution A (6.8 g/L of monobasic potassium phosphate in water, with pH 5.0 adjusted with 45% (w/w) solution of potassium hydroxide), and solution B (acetonitrile) (24:1, v/v). Flow rate: 1.5 mL/min. | UV (230 nm) | Amoxicillin, boluses, capsules, injectable suspension, for oral suspension, tablets, and tablets for oral suspension | USP 37 (2014) |
| Cylinder-plate | The microbiological assay described for other penicillin (Penicillin G) uses Staphylococcus aureus ATCC 29737 as test-micro-organism and culture medium 2 as base layer. | Not applicable | Intramammary infusion | USP 37 (2014) |
| Iodometry | Amoxicillin solutions were prepared in water, with adding NaOH, HCl and 0.01 M iodine, titrating with 0.01 M sodium thiosulphate and one drop of iodide starch paste. | Not applicable | Oral suspension | USP 37 (2014) |
| HPLC | L1 column (100 × 8.0 mm; 5 $\mu$m). Mobile phase: solution A (6.9 g/L monobasic sodium phosphate adjusted to a pH 4.2 with phosphoric acid) and solution B (methanol) (95:5, v/v). Flow rate: 2,0 mL/min. | UV (229 nm) | Amoxicillin and clavulanic acid extended-release tablets | USP 37 (2014) |
| HPLC | L1 column (100 × 8.0 mm; 5 $\mu$m). Mobile phase: solution A (6.9 g/L monobasic sodium phosphate adjusted to a pH 4.2 with phosphoric acid) and solution B (methanol) (95:5, v/v). Flow rate: 2.0 mL/min. | UV (229 nm) | Amoxicillin and clavulanic acid extended-release tablets | USP 37 (2014) |
| HPLC | $C_{18}$ column (250 × 4.0 mm × 5 $\mu$m). Mobile phase: solution A, (acetonitrile and buffer dihydrogen potassium orthophosphate, 1:99, v/v, with pH 5.0) and solution B (acetonitrile and buffer diidrogenio potassium orthophosphate, 20:80, v/v, with pH 5.0) (8:92, v/v). Flow rate: 1.0 mL/min. | UV (254 nm) | Amoxicillin sodium powder for solution for injection, amoxycillin trihydrate capsules, tablets and powder for oral suspension | European Pharmacopoeia (2013) |
| HPLC | $C_{18}$ column (250 × 4.0 mm; 5 $\mu$m). Mobile phase: solution A, (acetonitrile and buffer dihydrogen potassium orthophosphate, 1:99, v/v, with pH 5.0) and solution B (acetonitrile and buffer diidrogenio potassium orthophosphate, 20:80, v/v, with pH 5.0) (8:92, v/v). Flow rate: 1.0 mL/min. | UV (254 nm) | Amoxicillin sodium powder for solution for injection, amoxycillin trihydrate capsules, tablets and powder for oral suspension | British Pharmacopoeia (2012) |
| HPLC | $C_{18}$ column (300 × 4.0 mm; 10 $\mu$m). Mobile phase: sodium acetate trihydrate with pH 4.5 adjusted with acetic acid and methanol (95: 5, v/v). Flow rate: adjusted so that the Rt is about 8.0 min. | UV (262 nm) | Amoxycillin hydrate, and capsules | Japanese Pharmacopoeia (2011) |
| Cylinder-plate | Culture medium: number 11. Test-micro-organism: *Micrococcus luteus* ATCC 9341. | Not applicable | Amoxycillin trihydrate, capsules and powder for oral suspension | Brazilian Pharmacopoeia (2010) |
| Iodometry | Amoxicillin solutions were prepared in water, with adding NaOH, HCl and 0.01 M iodine, titrating with 0.01 M sodium thiosulphate and three drops of starch S.I. | Not applicable | Amoxycillin trihydrate, capsules and powder for oral suspension | Brazilian Pharmacopoeia (2010) |
| HPLC | $C_{18}$ column (300 × 4.0 mm; 3–10 $\mu$m). Mobile phase: potassium phosphate monobasic buffer with pH 4.4 adjusted with sodium hydroxide or phosphoric acid and methanol (95:5, v/v). Flow rate: 2.0 mL/min. | UV (220 nm) | Amoxicillin and clavulanate potassium powder for solution for injection, powder for oral suspension and tablets | Brazilian Pharmacopoeia (2010) |
| HPLC | $C_{18}$ column (300 × 4.0 mm; 3–10 $\mu$m). Mobile phase: potassium phosphate monobasic buffer with pH 4.4 adjusted with sodium hydroxide or phosphoric acid and methanol (95:5, v/v). Flow rate: 2.0 mL/min. | UV (220 nm) | Amoxicillin and clavulanate potassium powder for solution for injection, powder for oral suspension and tablets | Indian Pharmacopoeia (2007) |
| HPLC | C18 column (250 × 4.0 mm; 5$\mu$m). Mobile phase: solution A (acetonitrile and buffer dihydrogen potassium orthophosphate, 1:99, v/v, with pH 5.0) and solution B (acetonitrile and buffer diidrogenio potassium orthophosphate, 20:80, v/v, with pH 5.0) (8:92, v/v). Flow rate: 1.0 mL/min. | UV (254 nm) | Amoxicillin sodium powder for solution for injection, amoxycillin trihydrate capsules, tablets and powder for oral suspension | Portuguese Pharmacopoeia (2007) |
| HPLC | C18 column (250 × 4.6 mm; 5$\mu$m). Mobile phase: solution A (0.2 M potassium dihydrogen phosphate buffer with pH 5.0 and acetonitrile, 99: 1, v/v) and solution B (0.2 M potassium dihydrogen phosphate buffer with pH 5.0 and acetonitrile, 80:20, v/v) (92:8, v/v). Flow rate: 1.0 mL/min. | UV (254 nm) | Amoxycillin sodium | Argentina Pharmacopoeia (2003) |

HPLC: High performance liquid chromatography.

## Conclusion

Amoxicillin belongs to the class of penicillins and the subclass of aminopenicillins, and it is a bactericidal antimicrobial agent with broad spectrum of action. Considering that it is widely used worldwide, the development and validation of reliable analytical methods for its identification and quantification is highly relevant. It is critical to maintaining the characteristics of the active pharmaceutical ingredient and patient's safety. In addition, antimicrobials with lack of quality contribute to the emergence of the serious problem of bacterial resistance.

Even though there are numerous analytical methods for the analysis of this penicillin, most of them use toxic organic solvents and techniques not focused on Green Chemistry. Thus, it is of utmost importance to insert a progressive vision for implementing new environmentally friendly methods for the quality control of this antimicrobial agent, which also contributes to social and economical aspects.

## Conflict of interest

The authors declare no conflicts of interest.

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