ANALYTICAL SURVEY COMPARISON OF SOME BETA-LACTAM ANTIBIOTICS USED IN PRACTICE

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Abstract. Modern medicine has a vast set of antibacterial means - mainly antibiotics. They are the most frequently prescribed medicines in the therapeutic practice. The β-lactam antibiotics are most often indicated for prophylaxis and treatment of bacterial infections caused by susceptible organisms.

The analysis of these substances is a challenge due to their sensitivity and instability under different conditions. Keeping this in mind some chemical methods have often been applied as methods for quantitation. On the other hand, in an attempt to protect the structure, a number of instrumental methods have been developed, based on UV/VIS spectrometry and different chromatographic techniques.

Thus in this review we make an analytical survey comparison of the described in the literature methods for analysis of beta-lactam antibiotics, applied alone or in combinations, as antibacterial products.

Key Words: β-lactam antibiotics, analysis methods, survey;

Introduction

The beta-lactam antibiotics are an essential part in the treatment of infections.

Chemistry of β-lactam antibiotics

The β-lactam antibiotics consist of a four-membered β-lactam ring (B) fused with five-membered thiazolidine ring (A) for penicillins and six-membered dihydrothiazine ring for cephalosporins [1-4].

The structural formula of penicillins is presented on Fig. 1:

![Fig. 1. Schematic representation of the general structure of penicillins.](image)

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The penicillin molecule has three asymmetric C-atoms (C3, C5, C6), whereat the natural and the corresponding semi synthetic penicillins have the same configuration, concerning those centers [1, 4].

The general structure of cephalosporins is presented on Fig. 2:

![Fig. 2. Schematic representation of the general structure of cephalosporins.](image)

Fig. 2. Schematic representation of the general structure of cephalosporins.

The family of cephalosporin antibiotics is produced semi-synthetically by chemical attachment of side chains to 7-aminocephalosporanic acid. Cephalosporins have been classified as first, second, third
and fourth generation mainly on the basis of bacterial susceptibility patterns and resistance to β-lactamases. Table 1 represents the classification of cephalosporins according to their generation.

Based on their chemical structure and structure activity relationships and compared to penicillins, cephalosporins are characterized with decreased toxicity and more broad-spectrum [4].

Most β-lactam antibiotics work by inhibiting cell wall biosynthesis in the bacterial organism and are the most widely used group of antibiotics.

β-Lactam antibiotics are indicated for the prophylaxis and treatment of bacterial infections caused by susceptible organisms.

**Penicillins**

Mode of action: Penicillin and most other β-lactam antibiotics act by inhibiting penicillin-binding proteins, which normally catalyze cross-linking of bacterial cell walls (Fig. 3).

Penicillins remain the drug of choice in many pediatric infections, because in general they are well tolerated, safe, and efficacious against most bacterial agents affecting children and youngsters. On the other hand, the increasing resistance has limited the use of natural penicillins. Thus products with extended-spectrum, penicillinase-resistant derivatives, and combinations of penicillins with beta-lactamase inhibitors have been developed, which allowed their continued and broad application [5].

Staphylococci, such as Staphylococcus aureus, have developed resistance to the natural penicillins by the production of penicillinase. By modifying the β-lactam ring through an addition of an acyl side chain, semisynthetic penicillins have been constructed that are resistant to disruption by penicillinase. These penicillinase-resistant agents include oxacillin, nafcillin, dicloxacillin, and methicillin. Certain factors limit the use of these agents. For instance, methicillin is rarely used because of its association with interstitial nephritis [6]. Dicloxacillin has excellent bioavailability as an oral agent, but is poorly palatable. The emergence of methicillin-resistant S aureus, which is also resistant to the other semisynthetic penicillins, has also limited the use of these agents. Recently, however, many geographic areas are reporting increasing methicillin-resistance in their community-acquired S aureus isolates [7, 8].

The aminopenicillins (ampicillin and amoxicillin) were developed as broad-spectrum agents with activity against gram-negative bacteria and enterococci. Initially, they were active against many gram-negative strains of bacteria including Escherichia coli, Proteus spp, Salmonella, and β-lactamase-negative Haemophilus influenzae. Because of changes in susceptibility patterns in many geographic regions, however, aminopenicillins are no longer the drugs of choice for infections caused by many of these organisms.

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**Table 1. Classification of cephalosporins according to their generation.**

<table>
<thead>
<tr>
<th>First generation</th>
<th>Second generation</th>
<th>Third generation</th>
<th>Fourth generation</th>
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<tr>
<td>Cephalothin</td>
<td>Cefamandole</td>
<td>Cefotaxime</td>
<td>Cefepime</td>
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<td>Cepaparin</td>
<td>Cefuroxime</td>
<td>Cefitoxime</td>
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<td>Cefazolin</td>
<td>Cefonicid</td>
<td>Ceftriaxone</td>
<td>Cefcadin</td>
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<td>Cephalixin*</td>
<td>Ceforanide</td>
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<td>Cephradine*</td>
<td>Cefaclor*</td>
<td>Cefoperazone Cefixime*</td>
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<td>Cefadroxil</td>
<td>Cefoxitin</td>
<td>Cefpodoxime proxetil*</td>
<td>Cefpirome</td>
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<td>Cefotetan</td>
<td>Cefbiphenylbuten*</td>
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<td>Cefprozil*</td>
<td>Cefdinir*</td>
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<td>Cepuroxime axetil*</td>
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Streptococcus pneumoniae, the most common etiologic agent of otitis media, is an important example of the development of bacterial resistance to penicillin. Through production of penicillin-binding proteins with decreased affinity for penicillins, resistance of many strains of S pneumoniae has progressively increased. Because the mutations in the penicillin-binding proteins may result in only modestly reduced susceptibility, however, higher doses of aminopenicillins result in serum concentrations that exceed the MIC for a greater portion of the dosing interval, allowing effective killing [9].

With aminopenicillins as the prototypical extended-spectrum penicillin, other extended-spectrum groups were developed. These include the carboxypenicillins and ureidopenicillins.

Resistance to penicillins is mediated by a variety of mechanisms depending on the target organism. A primary mechanism of penicillin resistance is β-lactamase production. Inactivation of β-lactamases may be achieved by β-lactamase inhibitors, which irreversibly bind to the site of action and prevent hydrolysis of the penicillin. β-Lactamase inhibitors, however, have little antibacterial activity. The combination of β-lactamase inhibitors with certain penicillins has enhanced the selection of antibiotic agents available for pediatric patients. Amoxicillin-clavulanate is the most widely used combination agent in pediatrics. It is an excellent agent in the treatment of otitis media and sinusitis. [10]. The common adverse effect of gastrointestinal upset associated with clavulanate was found to be similar to the original formulation [10, 11].

Additional agents exist with limited indications in pediatric patients. Ampicillin-sulbactam is a parenteral combination that is approved for use in children 1 year of age and older. Its spectrum of activity is similar to amoxicillin-clavulanate. Indications include adenitis, pneumonia, and urinary tract infections. It has been used successfully in pediatric patients with bacterial meningitis [12], although its bactericidal activity in an experimental meningitis model was inferior compared with ceftriaxone [13]. It is also effective in treating pediatric bone and joint infections [14]. Ticarcillin-clavulanate is also approved for use in pediatric patients. It has been used successfully for empiric therapy in febrile neutropenic pediatric patients [15, 16].

**Cephalosporins**

**First-generation cephalosporins**

The two most widely used first-generation agents in pediatric patients are cefazolin, a parenteral agent, and cephalaxin, an oral agent. They are mainly used for skin and soft tissue infections caused by streptococci and susceptible S aureus. Both have proved records of safety and efficacy in children [5].

**Second-generation cephalosporins**

The second-generation cephalosporins have an enhanced spectrum of activity to include gram-negative bacteria. With the exceptions of the cephemycins (cefoxitin and cefotetan) they are not highly active against anaerobes. Additionally, this generation of cephalosporins has poor penetration across the blood-brain barrier. Cefuroxime is the exception with demonstrable penetration into the central nervous system. Its use for treatment of bacterial meningitis was associated with delayed sterilization of the cerebrospinal fluid, however, as compared with ceftriaxone and a higher incidence of hearing loss [17]. In addition to the parenteral formulation, cefuroxime is also available in an oral formulation (cefuroxime axetil) and is approved for use in children greater than or equal to 2 months of age. Other oral second-generation cephalosporins include cefaclor and cefprozil. Cefaclor, however, has been associated with serum sickness–like reactions that have limited its use in pediatrics [18]. Cefprozil has a spectrum of activity similar to cefuroxime axetil, yet is more palatable.

**Third-generation cephalosporins**

The most widely used parenteral third-generation cephalosporins in children are ceftriaxone, cefotaxime, and ceftazidime. These agents have enhanced activity compared with second-generation agents against the Enterobacteriaceae. In general, they have activity against penicillin non-susceptible S pneumoniae, Haemophilus, Neisseria, and Moraxella spp. They are ineffective against enterococci, Bacteroides, and Listeria. Their anti-staphylococcal activity is inferior, however, to the other generations of cephalosporins and semisynthetic penicillins. Other agents combined with third-generation cephalosporins may also be efficacious. Recently, cefotaxime has been shown to act synergistically with levofloxacin in an experimental meningitis model caused by penicillin-resistant S pneumoniae [19]. Cefotaxime has equivalent coverage compared with ceftriaxone and has no restrictive use for the newborn period. Ceftazidime is the only third-generation agent with activity against susceptible strains of P aeruginosa. Overall, these agents are well tolerated [20, 21]. Oral agents within this generation used in pediatrics include cefpodoxime, cefditoren, and cefixime.

**Fourth-generation cephalosporins**

The fourth-generation cephalosporins are dipolar ionic compounds that have a lower affinity for and are poor inducers of β-lactamases. They also dif-
fuse more rapidly into gram-negative bacteria. These characteristics are believed to lead to an overall lower development of resistance among gram-negative bacteria. Cefepime is the only approved agent in this generation. It has good activity against gram-positive bacteria including methicillin susceptible S. aureus, α-hemolytic streptococci, and some coagulase-negative staphylococci. Also, it has the best activity against penicillin-resistant pneumococcus among the cephalosporins [22, 23]. In addition, its gram-negative activity against H. influenzae, Neisseria spp, and Pseudomonas spp is excellent. Enterobacteriaceae are also included in cefepine’s spectrum of activity. Cefepime is approved for use in pediatric patients 2 months of age and older. It is indicated for treatment of febrile neutropenia, pneumonia, skin and soft tissue infections, and infections of the urinary tract [24, 25].

Analytical techniques for estimation of β-lactam antibiotics, used as therapeutic agents.

Analysis of these antibiotics is a challenge due to their sensitivity and instability in different conditions [26-32].

Chemical quantification

Based on the sensitivity and instability of the β-lactam antibiotics some titrimetrical methods for quantitation have been developed and applied. Most commonly the iodometric and mercurymetric titration have been used. These two methods are also presented in the Pharmacopoeia as methods for quantitation [33].

Iodometric titration – mechanism.

Iodometric titration of beta-lactam antibiotics is not based on oxidation of iodine directly, but on oxidation of the products of the hydrolysis of the beta-lactam structure (Fig. 4). The two products of hydrolysis – penadlic acid and penicillamine interact with the iodine molecule, applied as a titrant to form schiff base and 2-amino-3-sulpho-3-methyl-butanoic acid, respectively [34-36].

Mercury metric titration - mechanism

The mercury metric titration is the chemical method used for the determination of amoxicillin. The advantage of this method to others titrimetric methods is the obtaining of accurate results. The process is conducted in two stages (Fig. 5). On the first stage the analyzed substance is dissolved in a buffer solution and titrated directly with mercuric nitrate at room temperature, wherein the beta-lactam ring is opened. At the next stage, after full hydrolysis with an appropriate alkali, the substance is titrated again with Hg (II) – ions. In both cases the equivalent point is marked potentiometrically [1, 4, 33, 37-39].

\[
\text{Hg}^{2+} + 2\text{RSH} = \text{Hg(SR)}_2 + 2\text{H}^+
\]

\[
\text{Hg}^{2+} + \text{Hg(SR)}_2 = 2[\text{Hg(SR)}]^+ 
\]

Fig. 5. Schematic representation of the mercury metric titration.

Quantification of β-lactam antibiotics, using instrumental techniques.

UV/VIS spectrometry

A UV spectrophotometrical approach for quantification of penicillin antibiotics is presented. The method is based on measurement of the absorption of a complex obtained from interaction of the analyzed penicillin derivative with imidazole-mercury reagent (Fig. 6.) [1, 4].

Fig. 6. Quantitative determination of penicillins, using UV/VIS spectrometry

Resulting compounds absorb in the range 325-345 nm. The content of the analyzed product is calculated according to the single standard UV/VIS method [33, 39, 40].

Various UV spectrophotometric methods are reported for the analysis of some cephalosporin antibiotics are reported, alone and in presence of other drugs [30, 41, 42]. Using UV/VIS spectrometry Cefotaxime, ceftriaxone and ceftazidime have been determined in the presence of their alkali-induced degradation products, based on full spectrum quantitation over the range of 230-265nm [43].
Mixtures of ceftazidime, cefuroxime sodium, cefotaxime sodium and their degradation products have also been analyzed by first-derivative spectrophotometry at 268.6, 306, 228.6 nm, respectively. Additionally Cefotaxime and Cefuroxime have been determined through reaction with 1 chlorobenzotriazole followed by measurement of the absorbance at 298 nm [44-47].

Derivative spectrophotometry have also been applied for determination of some cephalosporins in binary mixtures [48-51]. A spectrophotometric method was reported for the determination of Cefalexin bulk drug and its acid-induced degradation products [52, 53].

Some first-derivative UV, second-derivative UV and H-point standard addition UV methods have been applied for the determination of Cefalexin in pharmaceutical preparations. For example a UV spectrophotometry and difference UV spectrophotometry have been applied to determine Cefalexin in tablets, and triethylammonium salt of Cefotaxime in the presence of related compounds resulting from the synthesis [54, 55].

Dissociation constants of Cefepime and Cefpirome have been determined by UV spectrometry. A UV spectrophotometric method has been applied for simultaneous determination of Cefuroxime axetil and probenecid in solid dosage forms [24, 53].

A derivative spectrophotometry has been reported for determination of Cefprozil in pharmaceutical dosage forms in the presence of its alkali induced degradation products.

Another method for determination of Cefadroxil in pharmaceutical dosage forms through mixing with sulfanilic acid has been reported, whereat the absorbance have been measured at 440 nm [50, 56].

**High performance liquid chromatographic methods**

The applications of HPLC for analysis of antibiotics is a powerful tool for therapeutic drug monitoring as well as clinical research [42, 46, 57-60].

In a lot of cases is essential to be able to determine the presence and quantity of more, than one β-lactam antibiotics. Recently a rapid and specific high-performance liquid chromatography method with UV detection for simultaneous determination of 12 beta-lactam antibiotics (amoxicillin, cefepime, cefotaxime, cefazidime, ceftriaxone, cloxacillin, imipenem, meropenem, oxacillin, penicillin G, piperacillin, and ticarcillin) in small samples of human plasma has been described. The method is easy to use in routine therapeutic drug monitoring, accurate and reproducible, and allows quantification of beta-lactam plasma levels from 5 to 250 μg/ml without interference with other common drugs [61].

As an answer to the need of a ways to detect possible cross-contaminations, a rapid RP-HPLC method for the simultaneous determination of 12 beta-lactam components for cleaning validation and cross-contamination has been developed. The method is specific with low limit of quantification, determined on the basis of the signal-to-noise ratio method and has been validated according to the International Conference on Harmonization (ICH) guidelines and has been used successfully for separation of 12 beta-lactam compounds within a run time of 50 min. The method has shown effectiveness for determination of cross-contamination of penicillin and cephalosporin [62].

Bacteria often develop resistance to β-lactam antibiotics by synthesizing a β-lactamase, an enzyme that attacks the β-lactam ring. To overcome this resistance, β-lactam antibiotics are often given with β-lactamase inhibitors such as clavulanic acid, sulbactam, tazobactam and brobactam [1-3, 63, 64].

This combined usage determines the necessity for analytical methods for identification and quantitation of such combinations. Thus in the literature some chromatographic methods are presented used for the analysis of different combinations carried out under different experimental conditions and varying some analytical parameters [65, 66].

The most common method for analysis of cephalosporins in formulations and in biological fluids is the HPLC method for analysis [67]. As reported in the literature several analytical procedures have been described for analysis Cefotaxime, Ceftazidime and Ceftriaxone in pharmaceutical formulations and biological fluids. These include high-performance thin layer chromatography [65, 68], high-performance liquid chromatography [69, 70], differential pulse adsorptive stripping voltammetry [71], NMR spectroscopy [72], polarography [73] and UV derivative spectrophotometry [74].

According to some literary data Cefradine and Cefalotin have been determined by spectrodensitometric method after contact with iodine vapors [75]. Cephalosporins were applied to TLC plates coated with a mixture (2:1) of layered double hydroxide of aluminum (III) and magnesium (II) and silica gel G and developed with a range of mobile phases, the spots were detected with iodine vapours and the cephalosporin content was determined [76, 77]. The degradation products of Ceftazidime, Cefuroxime sodium and Cefotaxime sodium after acid hydrolysis have been analyzed by quantitative densitometric TLC [78].

Ceftriaxone, Cefixime, Cefotaxime, Cefaclor and Cefalexin have been determined in their
pharmaceutical dosage forms using HPTLC and the measurement of each spot have been carried out at specified wavelengths using a scanner in absorbance/reflectance mode [79].

A method for simultaneous determination of Cefadroxil and Cefalexin in pharmaceutical preparations using quantitative TLC has also been reported [40, 68, 76].

In recent literature simple, accurate, precise and sensitive UV spectrophotometric and RP-HPLC chromatographic methods for simultaneous determination of Cefpodoxime and Clavulanic acid in combined tablets have been developed and validated. Both methods have been successfully applied for the analysis of the drugs in a pharmaceutical formulation and results of analysis have been successfully validated statistically and by recovery studies [80].

A review on the evidence of chemical structure and stability profiles of Ceftazidime [74] and Cefotaxime [81] has been reported. Recently, full spectrum quantitation (FSQ) has been used for rapid multi-component analysis of complex biological and pharmaceutical mixtures [82]. The technique has been successfully applied for simultaneous analysis of binary mixtures and ternary mixtures [42, 59, 83]. Moreover the use of FSQ and HPLC to quantify Cefotaxime, Ceftazidime and Ceftriaxone in the presence of their alkali-induced degradation products and in commercial injections [59, 84] has also been reported.

Furthermore some literary data demonstrate the application of the HPLC technique as a stability-indicating assay to study the kinetics of degradation of the beta-lactam antibiotics in aqueous solutions in various pH conditions [85-88].

**Biological assay**

The minimum amount of antibiotic, which is able to kill or inhibit the development of standard test-microbe in a defined culture conditions is considered to be a unit of biological activity [40]. This property has also been adopted as a method for determination of the chemotherapeutic features. For quantification of the biological activity the term Action Units is introduced [79].

**Conclusion**

A correct, fast and widely applicable method for analysis of penicillin and cephalosporin antibiotics alone and in mixture is an essential part for their application as antimicrobial agents. From the performed literary analysis may be concluded, that a number of methods are applied in order to assure the quality of the accepted beta-lactam antibiotics, used in treatment of microbial infections. The adopted and developed in the last years HPLC, RP-HPLC methods with number of detection techniques leave the impression for the most appropriate, when attempting to perform an accurate and fast analysis.

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