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Review paper

MICROBIOLOGICAL METHODS

Cephalothin: Review of Characteristics, Properties, and Status of Analytical Methods

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Abstract

Background: Cephalothin (CET), a first generation cephalosporin, is the most efficient cephalosporin against resistant microorganisms. Many studies found in literature and pharmacopeias propose analytical methods which are most commonly HPLC and microbiological assays.
Objective: This paper shows a brief review of analytical methods to quantify CET with a green analytical chemistry approach.
Method: The research data were collected from the literature and official compendia.
Results: Most of the analytical methods to determine CET were performed by HPLC and agar diffusion in pharmaceuticals, blood, urine, or water. Other analytical methods were found, such as UV-Vis, iodometry, fluorimetry, IR/Raman, electrochemical, and others in less quantity. One important aspect is that these methods use organic and toxic solvents like methanol and acetonitrile and only about 4% of the methods found use water as solvent.
Conclusions: Research about analytical methods for CET focusing on green analytical chemistry is of great importance and could optimize its analysis in pharmaceutical industries and help to guarantee the quality of the product. More than just the development of new techniques, it is possible to enhance the ones that already exist, applying the green analytical chemistry principles. In this way, it will be possible to reduce the environmental impacts caused by other analytical procedures.
Highlights: This work shows a brief review of literature and pharmacopeias of analytical methods to quantify CET. Its quality control can be updated to meet the needs of current analytical chemistry and to fit into sustainable and eco-friendly analysis.

In 1948, Professor Giuseppe Brotzu was able to isolate the *Cephalosporium acremonium* microorganism. From the obtained filtrate, he realized that there was an inhibition of *Staphylococcus aureus* growth. As a result, it was established that this microorganism is a producer of bacterial inhibitor substances (1). Until this moment, it was known that the filtrate was composed of three cephalosporins (N, P, and C), and the N and C cephalosporins showed a similar structure to penicillins (2, 3). In 1955, Abraham and Newton purified this filtrate, which allowed the isolation of C cephalosporin (1).

C cephalosporin showed weak antimicrobial action, but it was resistant to penicilinase enzymes, expanding its action spectrum. The modifications in lateral chains near to the cephalosporin's nucleus produced many new compounds for clinical use and it was of great importance in the treatment of bacterial infections (4).

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Cephalosporin Structure

Cephalosporins are antimicrobials, belonging to the class of beta-lactam antibiotics. From C cephalosporin, 7-aminocephalosporanic acid was isolated, a compound that has great action against microorganisms. Changes in this structure through addition of lateral chains made the production of semi-synthetic compounds, showing greater activity, possible (1, 4).

It is mainly divided, accordingly to its historical findings, spectrum of action and pharmacokinetics in generations (5). The first generation of cephalosporins shows the older ones and has great activity against Gram-positive cocci, limited activity against Gram-negative bacteria (*E. coli, Klebsiella,* and *Proteus mirabilis* are sensitive), and inactive against methicillin-resistant *S. aureus* (MRSA) and methicillin-resistant *S. epidermidis* (MRSE) (6).

Structure–Activity Relationship

Changes in the structure of cephalosporin can improve in vitro stability, antibacterial activity, and stability toward β-lactamases (7). The addition of an amino and a hydrogen to the α and α' positions, respectively, results in a basic compound protonated under acidic conditions of the stomach. The 7β amino group is essential for antimicrobial activity (X = H), and replacement of the hydrogen at C-7 with an alkoxy (-OR) results in an improvement of the antibacterial activity. The 6α hydrogen is essential for biologic activity and antibacterial activity is improved when Z is a five-membered heterocycle versus a six-membered heterocycle. Changes are usually made in positions C-3 and Y of the structure, which provides the different classes of cephalosporins (7, 8). Figure 1 shows the structure of cephalosporins and each position that can be changed.

Sodium Cephalothin

Sodium cephalothin (Figure 2) is an antimicrobial belonging to the class of first generation cephalosporins and has the molecular formula $C_{16}H_{15}N_2NaO_6S_2$. It is semi-synthetic and has great activity against Gram-positive microorganisms and less against Gram-negative (1). Cephalothin possesses an acetoxy group at position three of the molecule; as a result, cephalothin is metabolized to a compound, desacetylcephalothin, which has a much lower activity against both Gram-positive and Gram-negative bacteria (9).

Cephalothin is produced and commercialized in many countries around the world (10).

Mechanism of Action

The mechanism of action of cephalothin is related to inhibition of cell wall synthesis of microorganisms. This happens because of the binding to penicillin binding proteins (PBPs), responsible for the union of peptidoglycan units, so there is the impediment of the connection between structures. Consequently, the cell wall is not built, making the microorganism fragile and contributing to its death (2, 11).

Spectrum of Action

Cephalothin is active in the treatment of severe infections caused by Gram-positive aerobic microorganisms such as *S. aureus* (including beta-lactamases producer strains), *S. epidermidis* (including beta-lactamases producer strains), *Streptococcus pneumoniae, S. pyogenes*, and Gram-negative aerobic

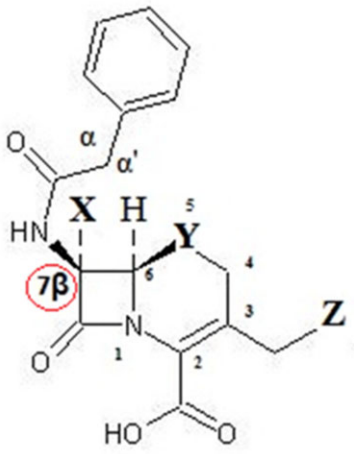

Figure 1. Structure of cephalosporins, indicating the β-position.

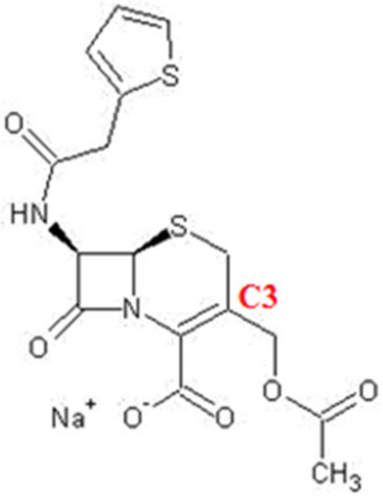

Figure 2. Chemical structure of sodium cephalothin (CAS 58–71-9) with emphasis on carbon 3.

microorganisms such as *E. coli*, *Haemophilus influenzae*, *Klebsiella* sp, *P. mirabilis*, *Salmonella* sp, and *Shigella* sp. It is indicated for the treatment of infections, from respiratory to gastrointestinal tract. Nowadays, it is used in prophylaxis in surgical treatments (12).

Polymorphism

A polymorph is a solid crystalline phase of a given compound resulting from the possibility of at least two different arrangements of the molecules of that compound in the solid state (13). In this way, each form can show different physicochemical characteristics and even cause changes in its effect.

The literature contains some studies that describe the development of polymorphs for cephalothin, but there are no reports of polymorphic forms for cephalothin anhydrate (14). It can be done through crystallization during the lyophilization procedure (14), recrystallization, freeze-drying (15), and grinding (15–16). The polymorphic forms found in cephalothin are basically crystalline (by grinding and recrystallization) and amorphous (freeze-drying) (15).

Physicochemical Properties

Cephalothin, as described above, is a semisynthetic first-generation cephalosporin designated as (6 R7 R)-3-(acetyloxymethyl)-8-oxo-7-[(2-thiophen-2-ylacetyl)amino]-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid. Its molecular formula and weight is $C_{16}H_{15}N_2NaO_6S_2$ (sodium salt) and 418.414 g/mol, and $C_{16}H_{16}N_2O_6S_2$ (anhydrous form) and 396.432 g/mol (17).

Cephalothin sodium occurs as white to light yellowish white crystals or crystalline powder. It is very soluble in water, slightly soluble in methanol, very slightly soluble in ethanol, and practically insoluble in acetonitrile (18). Its powder for injectable solution contains at least 90% and a maximum of 115% of sodium cephalothin (19) and pKa 2.22 (acid) (20) and after reconstitution, the solution has a pH of 6–8.5 (19).

Analytical Methods

There are some methods in the literature for the determination of cephalothin using microbiological assay (21–40), HPLC (26, 30, 41–48), HPLC tandem mass spectrometry (MS/MS) (49–50), iodometry (41, 51), TLC (51–54), UV (23, 29, 32, 41, 51), IR (23, 32, 53), IR-Raman (49), colorimetric methods (54–56), coulometry (52), NMR (52), differential pulse polarography (57), fluorimetry (58, 59), micellar electrokinetic capillary chromatography (60), first-derivative spectrophotometry (61), capillary electrophoresis (62), adsorptive stripping voltammetric technique (63), and electrochemical assay (36). The matrixes used were: dosage form (powder for injectable solution) (18, 19, 21–23, 27, 28, 30, 36, 41–58, 60, 62, 63, 88, 103, 115), animal and human blood and urine (25, 31, 58, 64–86, 114), peritoneal dialysate (87), artificial intestinal fluid (84), albumin protein (75–76), soil (31), and supply water (86).

Official compendiums show some methods for cephalothin in lyophilized powder for injection. The Brazilian Pharmacopoeia (19) describes three methods: HPLC, in the same conditions established by the United States Pharmacopeia (88), spectrophotometry in the ultraviolet region, using water as a solvent, and microbiological assay using the agar diffusion method. The United States Pharmacopeia (88) shows a monograph for sodium cephalothin in raw material and final product and proposes the use of HPLC. The European Pharmacopoeia (89) shows analysis for sodium cephalothin and related substances by HPLC. The Japanese Pharmacopoeia (18) describes an analytical methodology using HPLC.

Considering the importance of cephalosporin, this paper aims to review all analytical methods described in the literature for its analysis with a green analytical chemistry approach.

The methods shown in the literature for the determination of cephalothin are in Tables 1–7 and the methods described in official summaries are shown in Table 8. Figure 3 shows the distribution of analytical methods for cephalothin found in the literature and pharmacopeias.

Discussion

Among the analytical techniques found, the application of HPLC is the most prominent (Table 1), followed by microbiological assay (Table 3), especially in the last ten years.

Microbiological methods are greener when compared to those that use organic solvents. However, even in this case, it is possible to choose other options than the agar diffusion method. The turbidimetric assay is one of the best microbiological techniques in terms of green analytical chemistry. It has a smaller amount of steps and volume of work and uses less material, in addition to being faster (4 h compared to 24 h for the agar diffusion method) (90, 116, 117). All these characteristics make this method greener. Among the microbiological methods present in the literature for cephalotin evaluation, the turbidimetric technique was not found. The turbidimetric method can also be performed in miniaturized form (118), which is another option for the future analysis of cephalothin.

Another method widely used in the literature for the determination of cephalothin is HPLC (Table 1). This is a very selective method because it can separate mixtures that have a large number of substances (91). HPLC methods represent 45% of all methods found in the literature and all of them use methanol or acetonitrile in the mobile phase. None of them use ethanol, for example, which is a less toxic alternative and, as an organic solvent, may be a future option. Some methods also use buffer solutions (83.33%) in the mobile phase which increases the analysis time, because of the need for longer washing, and generates more waste. The injection volume varied between 10 to 100 µL and the flow rate between 0.4 to 5 mL/min. Considering the green analytical chemistry principles, lower volumes of solution and solvents used in analysis will be better for the environment and reduce the cost of analysis and waste generation. Another important parameter to be considered is the size of the column used during analysis. In the literature, they varied between 100 to 300 mm. A smaller column requires less solvent and the time of analysis is also shorter.

Some parameters included in green analytical chemistry can be followed using HPLC, such as the use of small and unspecified columns, which will reduce solvent waste and is consequently less expensive. The use of less toxic solvents and reduced flow rate improves conditions for the operator and reduces waste generation to the environment because the quantity of solvent used is smaller (92–102). A great many of the methods found in the literature have the use of buffer solutions in common. In these cases, it is necessary to prepare solutions more frequently and it is more expensive than using water. It is possible to develop a greener method and it has already been done (101). The use of less toxic solvents, such as ethanol and water, as well as the use of a smaller C18 column, makes this method eco-friendly. This new greener method produces great analytical results and the method can be used in the analysis of cephalothin, proving that the changes performed were effective and better for the environment, operator health, and costs. This chromatographic method could therefore become an interesting alternative for the analysis of this drug.

For antimicrobials, the association of physicochemical and microbiological methods is essential. Then, the cephalothin analysis must be completed by these two types of methods. The most ecologically correct suggestion is HPLC with green analytical chemistry principles and the turbidimetric microbiological method.

The comparison between HPLC and UV-Vis methods, shown in Tables 1 and 2, reveals that two UV methods use only water as diluents (32, 51). Depending on the intended purpose, this option is very interesting. While HPLC methods are the most common, they use toxic and disadvantageous but workable combinations in the mobile phase. The quantity of solvents used are higher than in the spectrophotometric method, so the UV method shows more advantages when compared to the chromatographic ones.

The IR method is the greenest technique among all those already developed for this drug because it does not use solvent

Table 1. Analytical methods for quantification of cephalothin by HPLC

Method	Conditions	Detection system	Matrix	References
HPLC	Column: reverse-phase C-18 μBondapak column (25 cm × 4.5 mm). Mobile phase: gradient which consisted of a 20 min linear gradient from 20 to 60% methanol 10 mM dibasic potassium phosphate buffer (pH 6.8). Flow rate: 1.0 mL/min.	254 nm	Powder for injectable solution	(46)
HPLC	Column: Zipax Sax DuPont® exchange column (100 × 30 mm); mobile phase: sodium acetate buffer solution 0.25 M (pH 5.0). At a flow rate of 0.82 mL/min, volume of injection of 74 μL.	254 nm	Urine (human)	(64)
HPLC	Column: stainless steel column (2 mm i) with a strong anion-exchange resin. The mobile phase was aqueous NaH_2P0_4 0.02 M adjusted to pH 8.5 with sodium hydroxide and a flow rate of 1 mL/min.	254 nm	Powder for injectable solution	(41)
HPLC	Deacetoxycephalothin: Mobile phase: acetate buffer 1 M pH 4.3 and a flow rate of 2.0 mL/min. [Acetyloxy)methyl]-2-[[[2-(2-thienylmethyl)-4-thiazoyl] carbonyl] amino]3-butenoic acid; mobile phase: K_2HPO_4 buffer 0.001 M pH 5.5 and a flow rate of 4.0 mL/min to which was added after 40 min of eluting time, a 0.43 M K_2HPO_4 solution at a rate of 10 mL/h to 1 L.	254 nm	Metabolites (deacetoxycephalothin and [acetyloxy) methyl]-2-[[[2-(2-thienyl-methyl)-4-thiazoyl] carbonyl] amino]3-butenoic acid).	(52)
HPLC	Column: stainless steel column (91.44 cm × 0.1778 in.), packed with AS-Pellionex-SAX resin; mobile phase: sodium dihydrogen phosphate 0.01 M containing 0.01 M of sodium nitrate (pH 4.8) and a flow rate of 26 mL/h. Urine: volume of 5 μL for the 1 h samples, 10 μL for the 2 and 3 h specimens, and 20 μL for later specimens. Serum: Aliquots of 40 μL were used for the 5 and 10 min specimens and 60 μL aliquots were used for later samples.	254 nm	Serum and urine	(66)
HPLC	Column: Phenyl Corasil® Reverse phase (90 × 2 cm); mobile phase: methanol: ammonium acetate buffer solution 0.2 M (10:90); flow rate of 2.0 mL/min; volume of injection 20 μL; retention time of 5 min.	254 nm	Human blood	(65)
HPLC	Column: aMicroBondapak C18 column (30 cm × 4 mm id). Mobile phase: methanol and 1% acetic acid (4:6) and a flow rate of 2.5 mL/min. The injection volume was of 100 μL and retention time of 9.9 min.	254 nm	Serum (human and dog)	(25)
HPLC	Column: Chromegabond C18 columns (4.6 mm × 30 cm) packed with 10 μm OX1.S. bonded phase; mobile phase: acetonitrile and 0.01 M monosodium phosphate solution where pH was adjusted to 5.0 with sodium hydroxide (85:15).	UV (wavelength not shown)	Susceptibility disks	(26)
HPLC	Column: reverse phase μBondapak C18. Mobile phase: methanol: 0.01 M sodium acetate buffer (pH 3.8) in a ratio of (35: 65) and a flow rate of 1.0 mL/min.	237 nm	Serum and urine	(67)
HPLC	Column: semipolar column (300 mm × 4 mm i). Mobile phase: 0.01 M NH_4OOCCH_3 in 30% methanol in water; with a flow rate of 2.2 mL/min and injection of 20 μL.	254 nm	Powder for injectable solution	(42)
HPLC	Column: Zorbax ODS® (150 × 4.6 mm); mobile phase: methanol: ammonium acetate buffer solution 0.2% (40:60); flow rate of 1 mL/min; volume of injection of 10 μL; retention time 12.0 min.	254 nm	Blood (rabbits) and urine (human)	(68)
HPLC	Column: μBondapak C18 (10 μm, 300 mm × 3.9 mm); mobile phase: methanol: sodium acetate buffer solution 0.01 M pH 4.8 (15:85); retention time of 8.0 min; flow rate of 1.5 mL/min and volume of injection of 10–15 μL.	240 nm	Plasma	(69)

(continued)

Table 1. (continued)

Method	Conditions	Detection system	Matrix	References
HPLC	Column: μBondapak phenyl column; mobile phase: acetonitrile, acetic acid, tertiary butyl ammonium hydrogen sulfate (25: 0.1: 0.3) in a flow rate of 2 mL/min.	254 nm	Plasma and urine	(71)
HPLC	Cephalothin was dissolved in 0.067 M phosphate buffer containing 0.15 M NaCI, pH 7.2. Column: gel permission type column model TSK-G2000SW. As mobile phase the same buffer was used; the flow rate was of 0.8 mL/min.	236 nm	Powder for injectable solution	(43)
HPLC	Column: a radialpack C_{18} column (8 mm × 100 mm); mobile phase: KH_2PO_4 0.02 M and methanol (60:40) and injection of 10 μL.	254 nm	Broth containing cephalothin	(104)
HPLC	Column: radially compressed C18 reversed-phase column Radial Pak C18, 8 × 100 mm, 5 μm particle size; mobile phase: monopotassium dihydrogen phosphate 0.1 M in doubly distilled water was adjusted to pH 4.0 (± 0.1) with phosphoric acid and acetonitrile (905:95, v/v) and a flow rate of 4 mL/min (retention time 25.74) or 5 mL/min (retention time 20.59 min).	254 nm	Powder for injectable solution	(44)
HPLC	Column: μBondapak C18 (250 × 4.7 mm), LiChrosorb RP-18 e Nucleosil C18 (150 × 4.7 mm); mobile phase: methanol: sodium acetate buffer solution 0.01 M (80:20); flow injection: 20–40 μL (plasma), 20 μL (milk and bile), and 10–20 μL (urine).	254 nm	Plasma, milk, bile and urine (human)	(70)
HPLC	Column: μBondapak C18 (10 μm, 300 mm × 4.0 mm); mobile phase: sodium acetate buffer solution 0.01 M pH 5.2: Acetonitrile (96%)-methanol (4%) (80:20); flow rate of 2.5 mL/min; volume of injection of 10 μL; retention time of 6.0 min.	254 nm	Plasma	(72)
HPLC	Column: 4.6 mm in internal diameter and 12.5 cm in length and filled with SC-02 was used. Mobile phase: ammonium acetate 0.2% and methanol (2:1, v/v)	254 nm	Plasma and urine (dogs)	(73)
HPLC	Column: reverse-phase micro-Bondapak phenyl column (300 mm × 3.9 mm i.d.). The mobile phase was ammonium acetate 0.01 M in either 25% or 35% aqueous methanol solution. The flow rate was of 1.8 mL/min.	UV (254 nm) and a Schoeffel variable wavelength UV detector set at 270 nm connected in series.	Powder for injectable solution (irradiated and not irradiated)	(45)
HPLC	This mixture was applied to a preconditioned 3 mL Baker SPE C_{18} extraction column. The column was then washed and the sample eluted with 0.5 mL of methanol. A 20 μL aliquot was injected.	ND[a]	Dialysate and serum	(74)
HPLC	Column: μBondapak C18 (300 × 3.9 mm); mobile phase: acetonitrile: ammonium acetate buffer solution 0.01 M (22:78); flow rate of 1.5 mL/min; volume of injection of 10–20 μL.	240 nm	Human albumin	(75)
HPLC	Column: Microsorb C8 5 μm (250 × 4.6 mm i.d.) fitted with a 15 × 4.6 mm i guard column of the same material. Mobile phase: acetonitrile and 5.5 mM octanesulfonic acid + 20 mM citric acid adjusted to pH 3.0 (28:72, v/v). The flow rate was 1 mL/min and the injection volume was 20 μL. The retention time was 7.2 min.	245 nm	Powder for injectable solution	(30)
HPLC	Column: μBondapak C18 (10 μm, 300 × 3.9 mm); mobile phase: methanol: phosphate buffer solution 0.06 M pH 7.4 (20–80%); flow rate of 1.5 mL/min.	270 nm	Human albumin	(76)

(continued)

Table 1. (continued)

Method	Conditions	Detection system	Matrix	References
HPLC	Column: Supelcosil C18 (150 × 4.6 mm, 3 μm) Supelco®; mobile phase: methanol: acetonitrile: phosphate buffer solution 0.01 M pH 7.0 (20:15:65) e tetrabutylammonium H_2SO_4 5 mM; flow rate of 1.0 mL/min; volume of injection of 10 μL; retention time of 13.0 min.	240 nm	Blood	(77)
HPLC	Column: μBondapak C18 (100 × 8 mm, 10 μm); mobile phase: acetonitrile: phosphate buffer solution 5 mM and acetic acid (22:77.5:0.5 v/v); flow rate of 2.0 mL/min; volume of injection of 180 μL; retention time of 13.6 min.	235 nm	Blood and tissue	(78)
HPLC	Column: Puresil® C18; mobile phase: acetate buffer solution 0.01 *M*: methanol (85:15).	270 nm	Blood and bronchoalveolar washing fluid (horses)	(79)
HPLC	Column: Microbore BAS C18 (150, 1 mm × 5 μm); mobile phase: methanol-acetonitrile: monossodium phosphate buffer solution100 mM pH 5.0 (20:20:60); flow rate of 0.05 mL/min; volume of injection of 10 μL; retention time 6.2 min.	254 nm	Blood (rats)	(80)
HPLC	Column: reversed-phase column (Tosoh TSK-GEL, 4.6 mm ×150 mm); the mobile phase was a mixture of phosphate buffer 50 mM (pH 2.5) and methanol (3:2).	260 nm	Powder for injectable solution	(47)
HPLC	Column: reversed phase C18 column (5 μm, 25 cm); mobile phase: phosphate buffer 20 mM (pH 4.4): methanol (72:28 v/v) at a flow rate of 1 mL/min.	254 nm	Intravitreal solution	(81)
HPLC	Column: (C-18) size was 250 mm × 5 mm and packed with amino (μNH2); mobile phase: formic acid, methanol, and water with a volume ratio of (0.5:0.5:99); flow rate was set to 1.0 mL/min and a retention time of 12.04 min.	254 nm	Reaction mixture prepared by the researcher	(105)
HPLC-MS/MS	Two mobile phase compositions were used: (A) 0.1% (v/v) aqueous HCOOH and (B) and 0.1% (v/v) HCOOH in CH_3CN; capillary temperature 180°C; sheath gas 60 psi, corona 4.5 A, and spray voltage 4.5 kV. The sample was dissolved in acetonitrile (1 mg/mL) and injected in a flow rate was of pure acetonitrile of 0.2 mL/min.	ND	Powder for injectable solution	(49)
HPLC	Column: Waters X-bridge C18 (300 × 4.6 mm, 2.5 μm); mobile phase: acetonitrile: sodium phosphate buffer solution 100 mM pH 3.0 (25:75, v/v); low rate of 1.0 mL/min; volume of injection of 25 μL; retention time of 2.1 min.	260 nm	Human plasma	(82)
HPLC	Column: ARC-Seibersdorf® 5C18 (250 × 4 mm); mobile phase: sodium acetate buffer solution 10 mM pH 5.9: acetonitrile: ethanol (78:15:07, v/v/v); flow rate of 1.0 mL/min; retention time of 5.0 min.	254 nm	Intestinal simulant fluid	(84)
HPLC	Column: Zorbax SB-C18 (150 × 4.6 mm); mobile phase: monobasic phosphate buffer solution 10 mM pH 4.8: methanol (gradient elution); flow rate of 1.0 mL/min; volume of injection of 50 μL.	240 nm	Soil	(31)
HPLC	Column: Waters X-bridge C18 (30, 4.6 mm, 2.5 μm); mobile phase: acetonitrile: sodium phosphate buffer solution 100 mM pH 3.0 (25:75); flow rate of 1.0 mL/min; volume of injection of 10 μL; retention time of 2.0 min.	260 nm	Human plasma	(85)
HPLC-MS/MS	Column: reversed-phase C18 analytical column (50 mm × 4.6 mm, 1.8 μm); mobile phase: solvents A: 10 mM formic acid in deionized water, B: 10 mM formic acid in methanol. A linear solvent gradient was used with a flow rate of 0.5 mL/min; volume of injection of 10 μL.	60 to 600 *m/z*	Powder for injectable solution	(50)
HPLC		238 nm		(48)

(continued)

Table 1. (continued)

Method	Conditions	Detection system	Matrix	References
	Column: Agilent Eclipse XDB-Phenyl (250 × 4.6 mm, 5 μm); mobile phase: sodium phosphate buffer solution 20 mM pH 4.5: acetonitrile (gradient elution); flow rate of 1.0 mL/min; volume of injection of 10 μL; retention time of 14.8 min.		Raw material and powder for injectable solution	
HPLC	Column: Zorbax SB-C18 (150 × 2.4 mm, 5 μm); mobile phase: water with formic acid 0.1%: methanol (60:40); flow rate of 0.3 mL/min; volume of injection of 20 μL.	ND	Supply water	(86)

[a] ND = Data not described.

Table 2. Analytical methods for quantification of cephalothin by spectrophotometry in the ultraviolet, visible, and infrared regions

Method	Conditions	Detection system	Matrix	References
Ultraviolet region				
UV	Extract four 30 μg disks with 1 mL water. Wash the disks with two additional 1 mL portions of water and collect all filtrates in a 5 mL volumetric flask. Dilute to 5 mL with water and record the UV spectrum.	220 to 310 nm	Antibiotic disks	(51)
UV	A solution of 2×10^{-4} M of cephalothin was mixed with a solution of phosphate buffer (6.7×10^{-3} M, pH 7.0) and a beta-lactamase (*B. cereus penicillinase*). All solutions having previously been allowed to equilibrate at 37°C. Changes in extinction were analyzed using a spectrophotometer.	260 nm	Dosage form	(23)
UV	Cephalothin was dissolved in acid, alkaline, or an appropriate buffer solution (for degradation in pH 10, 5 and 2) pre-heated at a desired temperature to produce a final cephalosporin concentration of about 5×10^{-5}–5×10^{-3}M.	260 nm	Powder for injectable solution	(41)
UV	Standard solution of cephalothin was transferred to produce working solutions in the range 4.0–32.0 μg/mL. Then, 5.0 ml buffer solution (pH 7.2) was added to each of the calibrated flasks and diluted to volume with distilled water. The absorption spectra were recorded using a 1.0 cm quartz cell.	235 nm	Standard material and dosage form	(60)
UV	Immersing plates that had been soaked in cephalothin for 15 min, 1 h, and 24 h in 8.2 mL of either water or PBS at room temperature. The release was measured in water for 1 h to investigate the initial release and then in PBS for 22 h.	239 nm	Release of the drug from coating implants (in water and PBS[a])	(29)
UV	Each specimen was immersed in 5 mL distilled water in a glass vial. The vials were stored at 37°C for 1, 2, 4, 7, 14, 30, and 60 days. At the indicated time intervals, the distilled water was collected to measure antibiotic concentration and then refreshed.	236 nm	Release from coatings	(32)
Visible region				
VIS	150 μL sample was added to 4.0 mL alkaline picrate reagent and allowed to stand for 8 min at ambient temperature. At the end of each time the absorbance was measured. The drug was dissolved in serum or phosphate buffer pH 7.4.	505 nm	Powder for injectable solution and serum	(54)

(continued)

Table 2. (continued)

Method	Conditions	Detection system	Matrix	References
VIS	Samples were transferred to a test tube, and water was added to make 4.0 mL and 2.0 mL of the nickel-hydroxylamine reagent and was added to the samples, which stood for 20 min. 1.0 mL 4 N HCI and 5.0 mL ferric chloride solution were then added.	490 nm	Powder for injectable solution	(55)
VIS	Cephalothin hydrolysis was performed using cephalosporinase from *C. freundii* GN 346 (in 0.1 M phosphate buffer pH 7.0) and alkaline (NaOH 0.2 N). The reaction was stopped by the addition of a phosphate buffer and 5 mL iodine reagent and in the alkaline hydrolysis.	540 nm	Powder for injectable solution	(103)
VIS	First adding one of the four different concentrations of alkaline picrate (20, 15, 10, and 7.5 mM) to each cuvette, then adding the creatinine or antibiotic.	500–520 nm	Powder for injectable solution	(56)
Chemiluminescence	Ratios of solution volumes of luminol: hydrogen peroxide cobalt (II): cephalothin of 0.8, 0.7, 0.03 were used.	420 nm	Powder for injectable solution	(115)
Infrared region				
IR	Cephalothin in the presence and absence of Neutrapen® (beta-lactamase) using Nujol as a vehicle on sodium chloride plates and a normal slit width and medium scan speed.	ND[b]	Dosage form	(23)
IR	The IR spectra taken in the solvent mixture consisting of 18% DMSO[c] in methylene chloride.	β-lactam band appeared at 1784 1/cm for deacetylcephalothin and at 1783 1/cm for cephalothin	Powder for injectable solution	(53)
IR- and Raman spectroscopy	Non-polarized solid-state IR spectra were recorded using the KBr disk technique at ambient conditions (T = 298 K, p = 1 atm). The oriented samples were prepared as colloid suspensions in nematic liquid crystals.	4000–400 1/cm, 0.5 1/cm resolution, 150 scans	Powder for injectable solution	(49)

[a] PBS = Phosphate buffered saline.
[b] ND = Data not described.
[c] DMSO = Dimethyl sulfoxide.

Table 3. Microbiological methods for assessing cephalothin potency

Method	Conditions	Detection system	Matrix	References
Microbiological assay	One tenth of a milliliter of a 1:100 dilution of a 24 h broth culture of the test organism was added to a series of tubes containing 2.0 mL brain heart infusion broth and cephalothin in concentrations of 5, 10, 20, 30, 40, and 100 μg/mL and MIC[a] was determined.	Absence of growth or zone inhibition	Powder for injectable solution	(21)
Microbiological assay	Two strains of *E. coli* and a strain of *P. mirabilis* (isolated from infected urine); the broth used was the complete broth of Lederberg; with an inoculum of about 10^4 CFU[c]/mL. The antibiotic dilutions were made using sterile distilled water. Serial two fold technique.	ND[b]	Powder for injectable solution	(22)
Microbiological assay	Cephalothin in a buffer solution pH 7.0 (25 μg/mL final concentration) at 37°C to determine the residual antibiotic activity after quenching enzyme action in aliquots by iodine. *Bacillus subtilis* NCTC[d] 8236 was used as a test organism. Agar cup-plate method.	Inhibition zones	Dosage form	(23)

(continued)

Table 3. (continued)

Method	Conditions	Detection system	Matrix	References
Microbiological assay	*S. aureus* NCTC 6571; 1 mL overnight 37°C broth culture was added to a 39 mL broth (pre-warmed to 37°C) containing the desired cephalothin concentration + Neutrapen®. Growth was measured in spectrophotometer due to changes in the color.	660 nm	Dosage form	(23)
Microbiological assay	0.2 mL overnight *S. aureus* cultures were added to a 10 mL broth containing various concentrations of a cephalothin + Neutrapen.	Presence/absence of growth	Dosage form	(23)
Microbiological assay	Various quantities of Neutrapen were added to the broth (final volume 10 mL) containing 50 mg cephalothin. After incubation, 0.1 mL (50–100 viable cells) of a 10^{-8} dilution of an overnight 30°C culture of *S. aureus* 6571 was added, the containers were incubated. Seven-day incubation at 30°C.	Presence or absence of growth	Dosage form	(23)
Microbiological assay	From a 10^{7} dilution of an overnight 37°C broth culture 0.1 mL was spread onto dried agar plates containing cephalothin ± Neutrapen. Incubation for 48 h at 37°C.	Colonies count	Dosage form	(23)
Microbiological assay	Trypticase soy agar was seeded with *Bacillus subtilis* spores. Standards were prepared by dissolving the antibiotic in serum obtained from each volunteer before the antibiotic was given.	Inhibition zones	Serum and urine	(24)
Microbiological assay	Microorganism: *B. subtilis* ATCC[e] 6633. Cephalothin was dissolved in distilled water alone at concentrations ranging from 0.625 to 10 μg/mL. Bacteria was inoculated and adjusted to a concentration equivalent to (2.4×10^{6} bacteria per mL).	Inhibition zones	Serum (human and dog)	(25)
Microbiological assay	Microorganism: *S. aureus* ATCC 13150; the method was performed by the standard methods described in the Code of Federal Regulations for ampicillin and carbenicillin.	Inhibition zones	Susceptibility disks	(26)
Microbiological assay	Microorganism: *S. aureus* (ATCC 6538-P); trypticase soy agar; three working dilutions of standard solution were made in concentrations of (0.5, 1.0, and 2.0 μg/mL of antibiotic). Phosphate buffer was used as a diluent.	Inhibition zones	Powder for injectable solution	(27)
Microbiological assay	22 *Y. kristensenii* strains; serial two-fold dilutions of the antimicrobial compounds (from 256 to 16 μg/mL) were incorporated into Mueller Hinton agar; the inoculum contained approximately 10^{5} CFU/mL.	Visual	Powder for injectable solution	(28)
Microbiological assay	Tester strains TA97a, TA100, and TA102 of *Salmonella typhimurium* with and without metabolic activation by S9 rat liver homogenate using the plate incorporation technique. A 100 μL portion of solution was added to each plate.	Count of revertants	Powder for injectable solution	(30)
Microbiological assay	*S. aureus* strain Cowan I; A Mueller–Hinton broth containing 0.8% agar was prepared and added approximately 5×10^{6} CFU/mL of *S. aureus* in a PBS suspension; the Petri dishes were then incubated at 37°C for 18 h.	Inhibition zones	Release of the drug from coating implants (in water and PBS[f])	(29)
		595 nm	Soil leavings	(31)

(continued)

Table 3. (continued)

Method	Conditions	Detection system	Matrix	References
Microbiological assay	*E. coli* K-12; 100 μL filtered supernatant and 100 μL 2 × Luria-Bertani medium with *E. coli* (10^6 mL) were added together in 100-well plates (5 wells per sample).			
Microbiological assay	*Streptococcus mutans* (IFO[g] 13955). The concentration of bacteria was adjusted to 10^6 –10^5 CFU/mL by dilution with a phosphate-buffered saline pH 7.2. Fifty microliters bacterial solution was pipetted. After 4 h, the specimen was rinsed with 1 mL PBS and bacterial cells were detached by sonication in PBS and 100 μL Fifty microliters harvested cells were plated onto Bacto-Agar plates.	Colonies count	Antibiotic in coatings	(32)
Microbiological assay	*S. aureus* (isolated from the nasal cavity of patients); the cultured colonies on blood agar were transmitted by a sterile loop to Mueller-Hinton agar media and antibiotic discs (30 μg) were put on them for 24 h at 35°C.	Inhibition zones	Antibiotic discs	(33)
Microbiological assay	*V. parahaemolyticus* (isolated from water, sediment, and shrimps). Around a 24 h old culture from the alkaline peptone water was spread as thick straight lines across the surface of pre-set and dried plates of the Mueller-Hinton agar. Antibiotic discs (30 μg) were placed onto the plates.	Inhibition zones	Antibiotic discs	(34)
Microbiological assay	*S. aureus* (isolated from nasal cavity of patients); the incubated colonies on blood agar were transported by a sterile loop onto the Mueller-Hinton agar, with the antibiotic containing discs (30 μg) placed on top.	Inhibition zones	Antibiotic discs	(35)
Microbiological assay	*Campylobacter* (isolated from stool specimens) were prepared in sterile saline and adjusted to a turbidity equivalent to a 0.5 McFarland standard and transferred onto the Mueller-Hinton agar plates. Inoculated plates were dried in an incubator for 5 min, and antibiotic discs were distributed.	Inhibition zones	Antibiotic discs	(37)
Microbiological assay	*E. coli* (isolated from water). One hundred microliters of the suspension (1×10^8 cells/mL) was then spread plated onto the Mueller Hinton agar plates and left to dry for 10 min prior to placing antibiotic discs (30 μg).	Inhibition zones	Antibiotic discs	(38)
Microbiological assay	*A. hydrophila*, *A. cavaie*, *A. sobria* (pig fecal samples); disc diffusion assays on the Mueller-Hinton agar using disc of cephalothin (30 μg).	Inhibition zones	Antibiotic discs	(39)
Microbiological assay	*E. aerogenes* ATCC 13084, *E. coli* ATCC 25922, *L. monocytogenes* ATCC 13932, *S. typhimurium* ATCC 13311, *S. aureus* ATCC 25923, *B. cereus* ATCC 11778, *S. epidermidis* ATCC 12228 and *B. subtilis* ATCC 6633. Tests were performed by the Mueller–Hinton agar-well diffusion method. The inoculum used was of 1×10^6 CFU and cephalothin discs (30 μg) were placed onto the plate.	Inhibition zones	Antibiotic discs	(40)

(continued)

Table 3. (continued)

Method	Conditions	Detection system	Matrix	References
Microbiological assay	One hundred and two *E. coli* strains (isolated from patient's urine) and *E. coli* ATCC 25922 (control); the test was carried out by disc diffusion method on the Muller-Hinton agar using antibiotic discs (30 μg).	Inhibition zones	Antibiotic discs	(106)
Microbiological assay	*S. aureus* (isolated from patients) and *S. aureus* ATCC 25923 (control); disc diffusion method in the Mueller-Hinton agar and using antibiotic disc of cephalothin (30 μg).	Inhibition zones	Antibiotic discs	(107)
Microbiological assay	Bacterial suspension was adjusted to 0.5 McFarland turbidity standards. The diluted bacterial suspension was then transferred to the Mueller-Hinton agar. After an incubation of 24 h, impregnated discs (30 μg) were placed on the plates.	Inhibition zones	Antibiotic discs	(108)
Microbiological assay	Seventy-nine pure isolated *E. coli* (from water) and *E. coli* ATCC 25922 (control); the 0.5 McFarland standard broth suspensions were inoculated on the entire surface of the Mueller-Hinton agar; antibiotic disc (30 μg) were placed onto the plates.	Inhibition zones	Antibiotic discs	(109)
Microbiological assay	*S. aureus* (from nasal infections) and *S. aureus* ATCC 25923 (control); disc diffusion method on the Mueller–Hinton agar using cephalothin (30 μg).	Inhibition zones	Antibiotic discs	(110)
Microbiological assay	*E. coli* (isolated from rain water), *E. coli* strain ATTC 25922 (positive control), and *E. coli* strain ATTC 35218 (negative control); 100 μL overnight *E. coli* culture was spread on the Mueller–Hinton agar; antibiotic disc containing 5 μg were placed onto the inoculated plates.	Inhibition zones	Antibiotic discs	(111)
Microbiological assay	*S. aureus* (from patients samples) and *S. aureus* ATCC25923 (reference); Mueller Hinton agar; the inoculum density was adjusted with 0.5 McFarland standard and spread on the plate and kept for 15 min before cephalothin discs containing 30 μg were added.	Inhibition zones	Antibiotic discs	(112)
Microbiological assay	*S. aureus* (from patients samples) and *S. aureus* ATCC25923 (reference); Mueller Hinton agar; the inoculum density was adjusted with 0.5 McFarland standard and spread on the plate and kept for 15 min before cephalothin discs containing 30 μg were added.	Inhibition zones	Antibiotic discs	(112)
Paper chromatogram	Whatman no. 4 paper (19 cm × 46.5 cm) impregnated with 0.1 M sodium acetate buffer (pH 4.6), methyl-ethyl-ketone (MEK), acetonitrile, and water (84:8:8) as a solvent system. The paper chromatogram was air-dried and placed for 15 min on an agar plate with 1% spore suspension of *Bacillus subtilis* ATCC 6633. After 15 min, the chromatogram was removed, and the plate was incubated at 37°C for 4 h.	Inhibition zones	Plasma supernatant, cerebrospinal fluid, synovial fluid or urine	(114)

[a] MIC = Minimum inhibitory concentration.
[b] ND = Data not described.
[c] CFU = Colony forming units.
[d] NCTC = National Colletion of Type Cultures.
[e] ATCC = American Type Culture Collection.
[f] PBS = Phosphate buffered saline.
[g] IFO = Institute for Fermentation.

Table 4. Analytical methods for assessing cephalothin by TLC

Method	Conditions	Detection system	Matrix	References
TLC	10 μg penicillin in a sintered-glass microfilter and pipet 1.0 mL methanol. Then collect the filtrate in a small flat-bottomed, evaporate the extract and redissolve in 50 μL methanol. Spot this solution on a TLC plate in 1.0 μL increments until 1.0 μg has been applied.	Visual	Antibiotic disks	(51)
TLC	TLC was performed on silica gel plates with a fluorescent indicator in a 16:1 acetone–glacial acetic acid system.	254 nm and iodine development	Powder for injectable solution	(52)

Table 5. Analytical methods for assessing cephalothin by fluorimetry

Method	Conditions	Detection system	Matrix	References
Fluorimetry	The aqueous solution of cephalotin was submitted to alkaline hydrolysis at pH 13 in NaOH (0.5 N) at 100°C for 30 min. After cooling, this solution was adjusted to pH 9 with HCl (0.5 N).	Fluorescence intensity: 430 nm; upon excitation: 380 nm	Powder for injectable solution	(58)
Fluorimetry	The dialysis was performed against a phosphate buffer M/15, pH 7.4, at 37°C for 3 h with constant stirring at 20 rpm[a]. Cephalothin concentrations were measured using the fluorimetry technique.	Fluorescence intensity: 430 nm; upon excitation: 380 nm	Human serum albumin	(58)
Fluorimetry	The spectra were corrected with quinine sulfate. The antibiotic concentration was always 1×10^{-4} mol/L and that of the metal ion 5×10^{-5} mol/L. The UV absorption spectrum of a kinetic mixture, cephalothin–Co^{2+}, was recorded until the end of the reaction at four different temperatures, 20, 25, 30 and 40°C.	UV (excitation: 330–362 nm; emission: 433–447 nm)	Methanolic solutions of antibiotic and mixture with metal ions (Cd^{2+}, Co^{2+} and Zn^{2+})	(61)

[a] rpm = Rotation per minute.

Table 6. Analytical methods for assessing cephalothin by iodometric assay

Method	Conditions	Detection system	Matrix	References
Iodometric assay	Two tests tubes with 30 μg sodium cephalothin. To both tubes add 1 mL 1% phosphate buffer (pH 6) and shake well. To one tube, add 1 mL 1 N sodium hydroxide, shake, and let it stand 15 min. Then add 1 mL 1.2 N hydrochloric acid and 0.2 mL 0.001 N iodine solution; after 15 min add 1 drop starch indicator solution. To the second tube add 2 mL distilled water, 0.2 mL iodine solution, and 1 drop starch indicator solution.	Visual	Antibiotic disks	(51)
Iodometric assay	From the degradation solution (5×10^{-3} M) two samples of 2 mL were pipetted into separate flasks. To the first sample, 5 mL 1 N NaOH was added. After standing for 20 min at room temperature, 5 mL 0.2 M phthalate buffer solution (pH 4.5), 5 mL 1 N HCl, and 10 mL 0.01 N iodine were added. Back-titration with 0.01 N thiosulfate. The second sample was treated with 5 mL pH 4.5 phthalate buffer solution and 10 mL 0.01 N iodine for 20 min in darkness at room temperature and then back-titrated with 0.01 N thiosulfate.	Visual	Powder for injectable solution	(41)

Table 7. Analytical methods for assessing cephalothin by other methods

Method	Conditions	Detection system	Matrix	References
Coulometry	Coulometric runs were made on solutions containing 500 mg cephalothin, per 80 mL and runs were made at three different buffered pH values: 3.1, 4.6, and 6.5, and one non buffered pH of 7–8.	ND[a]	Powder for injectable solution	(52)
NMR	The NMR spectra were recorded using $DMSO_{d6}$ as solvent and trimethylsilane as a calibrating standard.	C7 proton (5.5–5.7 ppm, multiplet), the C4 proton (5.12 ppm, singlet), the C4 proton (4.7 ppm, broad singlet), and the olefinic methine (8.36 ppm, singlet).	Metabolites (Δ^3-deacetoxy cephalothin, 3-exomethylene deacetoxycephalothin, β-isomer, 3-exomethylene deacetoxycephalothin, α-isomer and 3-[acetyloxy)-methyl]-2-[[[2-(2-thienyl-methyl)-4-thiazoyl] carbonyl] amino]3-butenoic acid).	(52)
NMR	Measurements were performed in d_6-DMSO at 22°C. The solvent effects have been measured in d_6-DMSO, d_6-acetone and in mixtures of these and the concentrations of compounds investigated were 0.03 *M*.	Data for 3-CH20 protons (acetone, δ, ppm): cephalothin: 5.093, 4.803	Powder for injectable solution	(53)
Micellar electrokinetic capillary chromatography	Fused-silica capillaries were conditioned by flushing capillaries 30 min with 1 M NaOH followed by deionized water for 10 min and carried out at 30°C. Hydrostatic injection times were 5 s. The applied voltage was 306 V/cm. Capillary distance to the detector was 50 cm.	210 nm	Capsules	(59)
Capillary electrophoresis	Fused-silica capillaries of 48.5 cm (length to the detector 40 cm × 50 μm i were used). The samples were injected at a pressure of 50 mbar for 9 s with a sample volume of 18.8 nL.	264 nm	Powder for injectable solution	(62)
Electrochemical assay	Solutions with 1 M HCl and 0.01 M cephalothin were prepared as stock solutions. Cephalothin solutions in different concentrations (5–200 ppm, mg/L). Electrochemical cell, using Ag-AgCl 3 M KCl reference electrode. The working electrode was immersed in the test solutions for 15 min.	Open circuit potential	Powder for injectable solution	(36)
Differential pulse polarography	Test solution was prepared from the Britton-Robinson buffer. The stock buffer solution (pH 2) was 0.04 M in boric acid, orthophosphoric acid, and glacial acetic acid: pH adjustments were made with a 0.2 M sodium hydroxide solution.	Forced drop time of 1 s, scan rate: 2 mV s^{-1} and pulse height of 50 mV	Powder for injectable solution	(57)
Adsorptive stripping voltammetric technique	Cephalothin stock solution of 1×10^{-2}M. Britton-Robinson supporting buffer (pH ≈ 2, 0.04 M in each constituent). The test solutions were purged with nitrogen for 8 min initially. The accumulation potential of 0.0 V versus Ag-AgCl was applied to a new mercury drop while the solution was stirred for 180 s at room temperature	Differential pulse	Powder for injectable solution	(63)

[a] ND = Data not described.

Table 8. Analytical methods for quantification of cephalothin—official summaries

Method	Conditions	Detection system	Matrix	References
HPLC	Column: C18 (250 × 4.6 mm, 5 μm); mobile phase: sodium acetate, acetic acid, acetonitrile, and ethanol (790:0.6:150:70 v/v/v/v), pH 5.9; flow rate: 1.5 mL/min; volume of injection: 10 μL.	254 nm	Powder for injection solution	(19)
HPLC	Column: C18 (250 × 4.6 mm, 5 μm); mobile phase: potassium phosphate pH 2.5 and acetonitrile (gradient mode); flow rate: 1.0 mL/min; volume of injection: 20 μL.	220 nm	Raw material	(89, 113)
HPLC	Mobile phase: sodium acetate, acetic acid, acetonitrile, and ethanol (790:0.6:150:70 v/v/v/v), pH 5.9; adjust the flow rate so that the retention time is about 12 min. Volume of injection: 10 μL.	254 nm	Raw material and lyophilized powder for injection solution	(18)
HPLC	Column: C18 (250 × 4.6 mm, 5 μm); Mobile phase: sodium acetate, acetic acid, acetonitrile, and ethanol (790:0.6:150:70 v/v/v/v), pH 5.9; flow rate: 1.0 mL/min; volume of injection: 20 μL.	254 nm	Raw material and lyophilized powder for injection solution	(88)
Microbiological assay	*S. aureus* ATCC 6538p; medium number two and prepare sample and standard solution to obtain 0.64; 1.0 and 1.56 μg/mL. Add 0.1 mL solution into the cylinders.	Inhibition zones	Powder for injection solution and raw material	(22)

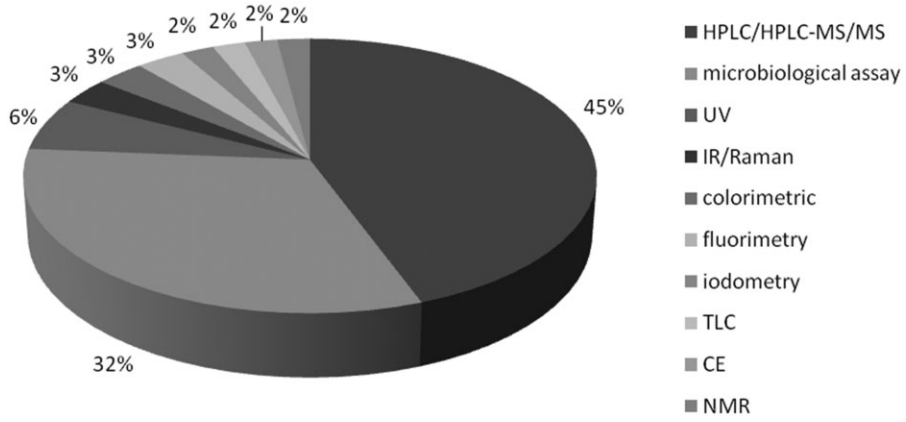

Figure 3. Distribution of analytical methods for evaluation of cephalothin. CE = capillary electrophoresis.

when it is performed using KBr (49, Table 2), and the generation of waste is reduced. It is also fast, provides results in a short time, and has easy handling. However, it is necessary to evaluate the intended use to ensure the suitability of the method.

Other options for the analysis of cephalothin are available in the literature, such as titration, capillary electrophoresis, electrochemical methods, and TLC. The use of one technique or another must be defined mainly based on the purpose of the analysis. The year that each technique was developed must also be taken into account as resources which are widespread today, may not have been available. Thus, the choice of methods depends on several factors. However, continuous improvement is always valid and can be applied to analytical techniques.

Conclusions

Cephalothin is an important antimicrobial belonging to the first generation of the class of cephalosporins and shows greater activity against Gram positive microorganisms, including beta-lactamase producers. This antimicrobial is used throughout the world and mainly for severe diseases. Therefore, it is very important to develop and validate analytical methods to guarantee their quality and safety for the patient. Besides the development of new techniques, it is possible to enhance the ones that already exist, applying the green analytical chemistry principle. By doing so, environmental impacts caused by analytical procedures can be reducted.

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Conflict of Interest

The authors declare no conflicts of interest.

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