IOSR Journal Of Pharmacy And Biological Sciences (IOSR-JPBS)
e-ISSN:2278-3008, p-ISSN:2319-7676. Volume 15, Issue 4 Ser. I (Jul. –Aug. 2020), PP 35-50
www.Iosrjournals.Org

Review on Bioanalytical Methods for Determination of Cephalosporins by Using HPLC and LC-MS

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Abstract:
Third- generation cephalosporins are semi-synthetic antibiotics initially derived from the fungus cephalosporium acremonium with enhanced activity against Gram-negative organisms. Serious health hazards are possible by internal control problems caused by its unstable structure,as well as food and environmental pollution introduced by improper use.the sensitive and valid methods for monitor and determination of cephalosporins in numerous matrices are required to beat the issues In recent years, numerous bioanalytical methods are developed to boost the sensitivity and specificity of determination of cephalosporins using the powerful LC-MS/MS systems that are common in research laboratories. This review aims to provide recently developed bioanalytical methods by HPLC or LC-MS(/MS) for third-generation cephalosporins from 1987-2019

Keywords: *third-generation cephalosporins; HPLC; LC-MS; LC-MS/MS; bioanalytical methods.*

Date of Submission: 13-07-2020 Date of Acceptance: 28-07-2020

I. Introduction:

Cephalosporins have become the most commonlyarbitrary β-lactam antibiotics since their first semi-synthetic production from cephalosporin C, the parent compound, in the 1940s (Klein and Cunha, 1995). Cephalosporins containing the 7-amino cephalosporonic acid nucleus and a six-membered dihydrothiazine ring fused to the lactam portion are one main class of Lactams (Weiqing Li et al ,2016)The presence of the four-membered β-lactam (2-azetidinone)7-ring is unique structural feature of β-lactam antibiotics, which is an essential group for biological activity (Xiao-Yi Duana et al.2019). Cephalosporins are consistently classified into first, second, third, fourth and now fifth-generation drugs, based on their spectrum of activity. After 10 years of use, the third-generation cephalosporins shows excellent antibiotics. They have superior activity against selected streptococcal species compared with other cephalosporins, and superior activity against Haemophilus, Neisseria and other less common oral gramnegative aerobic species (HAROLD C,1990).Third-generation cephalosporins have similar mechanisms of action to other β-lactam antibiotics, certain common Gram-negative organisms, such as Escherichia coli, Klebsiella, Citrobacterdiversus, Proteus and Morganella, are vulnerable to these drugs, and they are more active against Gram-negative bacilli compared with first- or second-generation cephalosporins (Barriere and Flaherty, 1984). The third-generation cephalosporinsare divergent. They are not only active against the major bacterial pathogens of infants and children, but they also achieve excellent cerebrospinal fluid bactericidal activity in experimental meningitis and in patients with meningitis

From a pharmacokinetic point of view , most parenteralthird-generation cephalosporins are inactivated in the stomach and have limited absorption from the duodenum, so oral administrationis not feasible, probably owing to their poor metabolic constancy, and are therefore applied clinically by intravenous and/or intramuscular injection. To avoid this problem, oral third-generation cephalosporins were developed, such as the methoximinic cephalosporins, which include cefixime, ceftibuten, cefpodoxime (proxetil) and cefetamet (pivoxil) (Novelli et al. (2000). even though third-generation cephalosporins do not show uniform pharmacokinetic properties, they are predominantly eliminated from the body by urinary or biliary excretion. In addition, many third-generation cephalosporins canpenetrate most body tissues and fluids well. For example,Protein binding is variable from 17% for ceftazidime to 96% forceftriaxone.41 (Yuk JH, Nightingale CH,R: Clinical pharmacokinetics of ceftriaxone. ClinPharmacokin 17:223, 1989)Ceftriaxone, however, even

though highly bound, achievesgood penetration into the cerebrospinal fluid.6, 33(Cherubin CE, Eng RHK, Norrby R, et al: Penetration of newer cephalosporins into cerebrospinal fluid. *Rev* Infect Dis 11:526, 1989)(33. Norrby SR: Role of cephalosporins in the treatment of bacterial meningitis in adults.Am J Med 79(suppl 2A):56, 1985)majority third-generation cephalosporins in various biological samples, such as plasma and urine, have been determined usinghigh-performance liquid chromatography (HPLC) and HPLC–massspectrometry (LC-MS) or tandem mass spectrometry (LC-MS/MS). In contemporary years, numerous bioanalyticalmethods have been developed to improve the sensitivity and specificity for quantification of third-generation cephalosporins, particularly as LC-MS/MS is now routinely used in pharmacokinetic experimental and clinical laboratories.To the best of our knowledge, however, there have been few reviews of bioanalyticalmethods using HPLC and LC-MS(/MS). A review in 1998 by Pehourcq and Jarry presented HPLC methods for quantification of some third-generation cephalosporins in biological fluids (Pehourcq and Jarry, 1998). However, as numerousexcellent bioanalytical methods using HPLC and LC-MS(/MS) have been reported over the past 10 years, an updated review of bioanalytical quantification methods is required.

Basis for Use of Third-Generation Cephalosporins(HAROLD C,1990)

- ✓ Tremendous activity against hemolytic streptococci
- ✓ Excellent activity against Haemophilus, branhamela, and Neisseria spp.
- ✓ Excellent activity against E coli, most Klebsiella, Porteus, Providencia and Serratia.
- ✓ More than adequate activity against S aureus.
- ✓ Acquiescent pharmacokinetics authorizing variable dosage programs depending on organismand patient.

Analysis of Cephalosporins:

There are various methods for the analysis of cephalosporins are reported in the various forms like chromatographic, UV, electrophoresis etc owing to their significance in clinical, pharmacological, and pharmaceutical studies .The applications of HPLC and LC-MS to the analysis of antibiotics introduce a powerful tool for therapeutic drug monitoring as well as clinical research 5. GC methods are fast but it requires elevated temperature, it may cause thermal degradation of drugs. To avoid that it requires derivatization to improve volatility chromatographic behavior. So these methods are not applicable for antibiotics. While other chromatographic methods having high limit of detection value so they are also not preferred. HPLC technique can provide valuable tool which generating high pure compound and has ability to analyze both volatile and nonvolatile compounds with ultra trace level may be employed in clinical research. Many antibiotics contain ionizable group canbe analyzed by ion exchange chromatographic methods. High resolving power of HPLC serves as a particularly important method for isolation and purification of antibiotics 5. Many methods for analyzing cephalosporins have been reportedowing to their significance in clinical, pharmacological, and pharmaceuticalstudies. Techniques, such as HPLC with ultraviolet(UV) or fluorescence (FL) detection and LC-MS(/MS), have generallybeen smeared for determination of cephalosporins in biologicalfluids (e.g. plasma, serum, bile and urine). However, thechemical instability of cephalosporins conceives complexities in theanalysis of cephalosporins, which is based on the chemical structureof the β-lactam nucleus with minor variationsin side chainsubstituents. Table 1 shows pKa and salt forms of oral and parenteral thirdgenerationcephalosporins mentioned in this review. The HPLCand LC-MS(/MS) methods used to analyze third-generationcephalosporins aregivenin the following

HPLC DETERMINATION OF THIRD-GENERATION CEPHALOSPORINS

Cephalosporins are usually analyzed byHPLC-based methodsin biological fluids; the parameters that are to be optimized are sensitivity,specificity and precision is still in progress. Because the comprehensive review paper by Pehourcq andJarry (1998) already illustrated the bioanalytical HPLCmethods for each third-generation cephalosporins, bioanalytical methods for these agents are not discussed here individually in terms of HPLC and biological sample preparation(See Pehourcq and Jarry, 1998). Instead, Tables 2 and 3 presentsummaries of the developed HPLC methods with definite conditions(e.g. sample preparation, column, mobile phase and detection for individual and simultaneousdetermination of third-generation cephalosporins, which havebeen developed since the 1980s, respectively. We more focuson the simultaneous determination of HPLC methods of third generation cephalosporins in this section rather than individual methods.As shown in Table 3, based on the stability and polarity of the drug the HPLC-based techniquesare reported for simultaneous determination of third-generation cephalosporinsmainly differ with regard to sample preparationmethods, with protein precipitation using solvents, such as acidor solvents of different origins, and solid-phase extraction (SPE). In addition,the ionic strength and pH of the mobile phase were found tobe important factors affecting the analytical conditions and results.In general, most HPLC-based bioanalytical methods forcephalosporins use an acidic eluent of low ionic strength basedon the chemical structure and buffer conditions (Pehourcq andJarry, 1998).administration in various clinical situations. Therefore, the simultaneousmonitoring of two to seven third-

generation cephalosporinscan be routinely conducted in the same injectiontogether with other antibiotics (up to 15), such as penicillinsand second-generation cephalosporins, depending on the characteristicsof the cephalosporins.

Khan et al. (2011b) reported a simultaneous bioanalyticalHPLC-UV method for cefdinir and cefixime in human plasma.In this method, human plasma samples were simultaneouslyanalyzed on a C18 (150 × 4.6 mm, 5 μm) column using a mobile phase of acetonitrile, methanol (MeOH; 50:50, v/v) and 0.05%trifluoroacetic acid (TFA) after denaturation of plasma protein,and solvent extraction of cefdinir and cefixime. The HPLC-UVmethod developed by Khan et al. had sufficient sensitivity tomonitor cefdinir and cefixime in plasma (limit of detection, LOD, 1 ng/ml; LOQ, 4 ng/ml) and a high resolution (retentiontime, 2.4 min for cefdinir and 3.8 min for cefixime; total runningtime, 5 min), and has been successfully applied in pharmacokineticstudies.

Cefotaxime and ceftizoxime were alsoanalyzed together in human serum, urine and blister fluid by HPLC-UV (Vallee andLeBel, 1991). In sample preparation, acetonitrile was used forprotein precipitation and methylene chloride was used fordelipidation. The use of acidic conditions in the sample preparationsteps should be avoided for analysis of cefotaxime andceftizoxime because of stability issues. Similarly, ceftizoximeand the first-generation cephalosporin, cefazolin, were simultaneouslydetermined in human serum by HPLC-UV (Arayneet al., 2007b).

For the simultaneous determination of ceftibuten, cefixime,cefuroxime and the second-generation cephalosporin, cefaclor,the cephalosporins were analyzed together in human plasmausing a sensitive HPLC-UV method in a pharmacokinetic study(Nix et al., 1997a). In addition, third-generation cephalosporins(cefotaxime, cefoperazone, cefmenoxime, ceftazidime and ceftriaxone)and other antibiotics (benzylpenicillin, ampicillin, cloxacillin,ticarcillin, mezlocillin, azlocillin, piperacillin, cefsulodin, andthe monobactam, aztreonam) were successfully analyzed by Jehlet al. (1987a) with a simple sample preparation step (i.e. proteinprecipitation) and dilution owing to their high polarity. For routinemonitoring of the concentrations of many antibiotics in hospitalsettings, a high-speed analytical column (75 × 4.6mm) filled withoctadecylsilane-coated silica particles (3 μmin diameter) was used,owing to their shorter retention time and rapid analysis.

Karageorgou et al. (2012b) also reported the simultaneousanalysis of seven cephalosporins, specifically third-generationcephalosporins (cefoperazone, cefixime, ceftazidime, ceftizoxime,ceftriaxone and cefotaxime) and the fourth-generation cephalosporin,cefepime, in human plasma and amniotic fluid using anHPLC-UV method (Karageorgou et al., 2012b). Karageorgou et al.also described the simultaneous analysis of certain third-genecephalosporins, including cefoperazone, ceftiofur and cefotaxime, and other antibiotics (cloxacillin, dicloxacillin, oxacillin, amoxicillin,cefaclor, cefadroxil, cefuroxime, cefazolin and cephalexin) in milk byHPLC-UV

(Karageorgou et al., 2012b). In these cases, SPE was usedfor sample preparation based on the polarity and instability of cephalosporinsin biological fluids. To separate many drugs in the sameinjection, gradient elution methods of mobile phase were introducedat various detection wavelengths. In addition, Verdier et al. (2011)reported the analysis of 12 antibiotics, including third-generationcephalosporins (cefotaxime, ceftazidime and ceftriaxone) and otherantibiotics (amoxicillin, cefepime, cloxacillin, imipenem, meropenem,oxacillin, penicillin G, piperacillin and ticarcillin). For sample preparation,protein precipitation with acetonitrile and delipidation with chloroformwere performed for the robust and validated analysis of drugs.

Sun et al. (2012b) described an HPLC-UV method for analyzing ceftriaxone and other antibiotics (metronidazole and levofloxacin) inhuman urine. For the robust analysis of these drugs, protein precipitationwith acetonitrile was first performed before injection into theHPLC column to maintain the stability of cephalosporins based onthe polarity of ceftriaxone.

Shah et al.(2013) described an HPLC-UV method for simultaneous determination of Ceftriazone and Cefaclor in commercial formulations and biological samples. For analysis of these drugs ,Column C18 (250 mm × 4.6 mm; 5 mm); at ambient temperature.Mobile phase used is acetonitrile, methanol and triethylamine (TEA) buffer(pH 7) (1:1:2 v/v), flow rate 0.6 ml/min, injection volume 20 ml.

Raveendra et al (2019) described a novel approach to develop and validate a bioanalytical RP-HPLC method for the simultaneous estimation of Paracetamol and Cefixime in rabbit plasma using Cefaclor as internal standard. Evaluation of the drugs content were done by a mixture of Phosphate buffer (p^H 6.4) and Acetonitrile (80:20, v/v) as the mobile phase and measure the absorbance at 245 nm for Paracetamol and Cefixime. Retention time established to be 3.618 min for Cefaclor, 4.608 min for Paracetamol and 5.914min for Cefixime. The results shown that the analytical technique furnished here establishes acceptable accuracy and precision, shorter and easy sample preparation, reduced the complications for equipment on satisfactory analysis time.Calibration curves were plotted in the concentration range of 10-100μg/ml and 5-50 μg/ml for Paracetamol and Cefixime respectively of required concentrations in the measured samples

.LC-MS(/MS) determination ofthird-generation cephalosporins

To the best of our knowledge, there have been one previousreview regarding LC-MS(/MS) bioanalytical methods used fordetermination of third-generation cephalosporin antibiotics inbiological samples. Here, we describe methods used for the quantitativeanalysis of cefdinir, cefetamet, cefixime, cefpodoxime, ceftibuten,cefoperazone, cefotaxime, ceftazidime and ceftiofur by LC-MS(/MS), asshown in Table 4. Unfortunately, there have been no reports regardingLC-MS(/MS) analysis of cefmenoxime and ceftizoxime.

Cefdinir

Two reported bioanalytical methods based on LC-MS/MS for quantificationof cefdinir from biological fluids (rat plasma/urine and humanplasma) involved deproteinization of biological samples with either10% trichloroacetic acid (TCA) (Chen et al., 2006) or MeOH (Jin et al.,2013). Chen et al. (2006) and Jin et al. (2013) used other cephalosporins,cefaclor and cefadroxil, respectively, as an internal standard (IS)for quantification in LC-MS/MS detection. Reverse-phase (RP) analyticalcolumns (C18)were used by both groups for chromatographic separationof cefdinir. For quantification of cefdinir in human plasma, thestandard curve of cefdinir ranging from 5 to 2000 ng/ml was obtainedusing HPLC-MS/MS by selected reaction monitoring (SRM) inpositive mode (m/z 396.1→m/z 226.9; Chen et al., 2006).

On the other hand, quantification of cefdinir in rat plasma and urine samplesshowed a relatively broad concentration range of standard curves(10–10,000 ng/ml) using LC-MS/MS in positive mode (m/z396.1→m/z 227.2; Jin et al., 2013). Particularlly , the issue of stabilityof cefdnir in rat plasma and urine at room temperature for 24 h hasbeen raised and careful handling may be required at room temperature(Jin et al., 2013).

cefetamet

CefetametNoh et al. (2011) validated the quantitative determination ofcefetamet in human plasma by HPLC-MS. Two other studiesanalyzed cefetamet as an IS for quantitative analysis of cefixime(Meng et al., 2005) and cefaclor (Chen et al., 2003) in humanplasma using HPLC-MS/MS. Plasma samples were processed by protein precipitation (Meng et al., 2005; Noh et al., 2011) orSPE (Chen et al., 2003), and a wide range of analytical columns(C8 and C18) for chromatographic separation of cefetamet werechosen. Noh et al. (2011) established an HPLC-MS method inpositive mode that provided a calibration curve for cefetametranging from 5 to 5000 ng/ml (m/z 398.1) in human plasma(Noh et al., 2011). Two other previously reported LC-MS/MSmethods were based on SRM (m/z 398→m/z 241) scans in tandemmass spectrometry (Chen et al., 2003; Meng et al., 2005).

Cefixime

Methods for quantification of cefixime in human plasma wereestablished using HPLC-MS (Attimarad and Alnajjar, 2013) andHPLC-MS/MS (Meng et al., 2005). Both groups used deproteinization methods for quantificationof cefixime in human plasma, with moxifloxacin(Attimarad and Alnajjar, 2013) and cefetamet (Meng et al.,

2005) as an IS, respectively. Attimarad and Alnajjar (2013) useda C18 column with isocratic elution of the mobile phase and obtained a linear calibration curveranging from 40 to 6000 ng/ml cefixime (m/z 453.8) by HPLC-MSin positive mode. Meng et al. (2005) used a C8 column withisocratic elution of the mobile phase for chromatographic seperation and provided a linearcalibration curve for cefixime over a broad concentration range(50–8000 ng/ml) by HPLC-MS/MS based on SRM (m/z 398→m/z 241)in positive mode.

Cefpodoxime

There has only a single report regarding analysis ofcefpodoxime in biological fluid (human plasma) by LC-MS(Dubala et al., 2013). Cefpodoxime in human plasma was quantifiedby SPE with HPLC-APCI-MS using chloromophenicol as I.S.A Princeton SPHER C18 column was used for chromatographicseparation of plasma samples. A standard linear curveranging from 0.04 to 4.4 µg/ml was obtained, and the quantificationof cefpodoxime was performed using selected ion monitoring(SIM) mode in negative mode at m/z 408, owing to itsselectivity (Dubala et al., 2013). LC-MS/MS method hasnot been applied for quantification of cefpodoxime.

Ceftibuten

We found only a single reference regarding determination ofceftibuten by LC-MS from biological fluids, and it involved analysisof ceftibuten in both human sputum and plasma (Pan et al.,1993). The authors quantified ceftibuten (0.50–10.00 µg/ml)from human sputum using a simple dilution with 0.1 M ammoniumacetate solution and mass spectrometric detection withthe thermospray (TSP) technique. The LC-LC-TSP-MS assay witha Waters µBondapak phenyl column provided much better selectivity(m/z 226) than the previously reported LC-LC-UVmethod (Pan et al., 1992), although there was no advantage insensitivity. The stable isotope-labeled IS is the bestchoice for quantitative LC-LC-TSP-MS. However, a stableisotope-labeled

cis-ceftibuten standard was not available, sothe authors used the direct external standard calibrationmethod (Pan et al., 1993).

ceftriaxone:

There has only been a single report regarding analysis of ceftriaxone in biological fluids, by LC-MS (Mariana Teixeira da Trindade,2018).ceftriaxone in blood was determined by UPLC-MS/MS ColumnC18 Wwaters acquity T3 (50 mm × 2.1 mm; 1.7 µm). Mobilephase: 0.1% formic acid in water and 0.1% formic acid inacetonitrile (gradient mode); flow rate 0.4 ml/min; injectionvolume 5 ml.Positive electrospray ionization(ESI) using multiple reactionmonitoring (MRM) in Blood(Page-Sharp, M.; et al,2016).

Cefoperazone

Quantitative determination of cefoperazone in human plasma(Tsujikawa et al., 2008; Zhou et al., 2010) and milk (Hou et al.,2013; Junza et al., 2011; Li et al., 2014) was investigated usingLC-MS(/MS). For human plasma samples, protein precipitation(Tsujikawa et al., 2008) and liquid–liquid extraction (Zhou et al.,2010) were used for sample pre-treatment, and cefoperazonewas separated on a C18 column (Tsujikawa et al., 2008; Zhouet al., 2010). Calibration curves for cefoperazone were linear overthe range 0.07–1.93 µg/ml in HPLC-MS by SIM in positive mode(m/z 646; Tsujikawa et al., 2008), and 0.1–20 µg/ml in HPLC-MS/MS by SRM in negative mode (m/z 644.0→m/z 115.0; Zhouet al., 2010). For bovine milk samples, after liquid–liquid extraction(Li et al., 2014), SPE (Hou et al., 2013; Junza et al., 2011; Liet al., 2014) and SPE with C18 (Liu, et al., 2014) for sample processing, cefoperazone was separatedfrom endogenous peaks using C8 (Junza et al., 2011) and C18(Hou et al., 2013; Junza et al., 2011; Li et al., 2014; Liu et al.,2014) columns. By using HPLC coupled with tandem mass spectrometry detection(MS/MS) cefoperazone analysis was done by multiplereaction monitoring mode (MRM) in positive mode and linearity was obtained over the rangeof 0.5–1.25 µg/kg (m/z 646→m/z 290; Junza et al., 2011) and 2–5000 ng/ml (m/z 530; Liu et al., 2014)Ultraperformance liquidchromatography (UPLC)-MS/MS was also applied to analyzecefoperazone in positive mode (Junza et al., 2011; Hou et al.,2013), and both positive and negative modes (Li et al., 2014).Calibration curves for cefoperazone in milk were 5–150 µg/kg(m/z 646→m/z 290; Junza et al., 2011), 2–250 µg/kg(m/z 646.45→m/z 143.05; Hou et al., 2013), and 1–100 µg/L(m/z 644.0→m/z 115.0; Li et al., 2014) using the UPLC-MS/MS system.

Cefotaxime

There have been two recent reports of UPLC-MS/MS analysis ofcefotaxime (Hou et al., 2013;ss Li et al., 2014). Validations of bothmethods for cefotaxime by UPLC-MS/MS were established usingmilk samples, but not from other biological fluids, such asplasma and urine, on mass spectroscopy. Hou et al. (2013) usedceftiofur-D3 as an IS, processed milk samples using SPE, and separatedcefotaxime from endogenous peaks on a universal C18column for UPLC with gradient elution. Calibration curves rangingfrom 2 to 250 µg/kg of cefotaxime (m/z 456.43→m/z 396.16)in milk were evaluated for linearity by UPLC-MS/MS in positivemode (Hou et al., 2013). Li et al. (2014) quantified cefotaxime(2–100 µg/l) from bovine milk after sample processing byliquid–liquid extraction and SPE. Cefotaxime was separatedusing a C18 column and could be detected by UPLC-MS/MS inboth negative and positive modes (m/z 454.0→m/z 239.0), butshowed a higher response in negative mode (Li et al., 2014).

Ceftazidime

Methods for quantitative determination of ceftazidime haveonly been established in human plasma using UPLC-MS/MS(Carlier et al., 2012; Colin et al., 2013) and HPLC-MS/MS analyses(Sime et al., 2014). In UPLC-MS/MS analysis, SPE (Carlier et al.,2012; Colin et al., 2013), protein precipitation, and liquid–liquidextraction were used for sample processing. The calibrationcurve linearity of ceftazidime in human plasma was set as0.5–100 µg/mL with an HSS T3 column (Colin et al., 2013) andas 0.76–90.81 µg/mL with a C18 column (Carlier et al., 2012). Ceftazidime was detected at m/z 547.1 of the parent drug (Colin et al., 2013) and m/z 547.22→m/z 468.10 of a fragment (Carlieret al., 2012) on UPLC-MS/MS by MRM in positive mode. Sime et al. (2014) performed quantification of ceftazidime in human plasma using HPLC-MS/MS by MRM in positive mode.

Samples were processed by simple protein precipitation withacetonitrile containing 0.1% formic acid, and were separatedon a C18 column; a fragment of ceftazidime was detected atm/z 547.2→m/z 167.1 for quantification (Sime et al., 2014).

Ceftiofur

Quantitative determination of ceftiofur in milk was performed by HPLC-MS/MS (Daeseleire et al., 2000; Junza et al., 2011) and UPLC-MS/MS (Hou et al., 2013; Junza et al., 2011). However, ceftiofur has not

been analyzed in other biological fluids (e.g. plasma, urine). Daeseleire et al. (2000) used nafcillin as an IS, and separated ceftiofur in retail and farm milk on C18 columns after simple protein precipitation with acetonitrile. On HPLCMS/ MS in positive mode, the LOD and LOQ of ceftiofur(m/z 524→m/z 241) in retailmilk were 1 and 3 μg/kg, respectively, while those in farm milk were 3 and 6 μg/kg, respectively. Junzaet al. (2011) also reported the determination of ceftiofur in milk using HPLC-MS/MS; they developed and validated a bioanalyticalmethod for determine ceftiofur in cow's milk by HPLC-MS/MS and UPLC-MS/MS with MRM (m/z 524→m/z 285) in positive mode, and these techniques were also compared. Pipemidic acid was used as an IS, and milk samples were processed by protein precipitation with acetonitrile followed by SPE with different cartridges (Oasis HLB and Strata X cartridges). The LOD and LOQ of ceftiofur with the C8 column in HPLC-MS/MS (0.3 and 0.5 μg/kg, respectively) were lower than those with the C18 column in UPLC-MS/MS (0.75 and 2.5 μg/kg, respectively). Similarly, Hou et al. (2013) reported UPLC-MS/MS analysis for the determination of ceftiofur in milk. Ceftiofur was extracted using SPE cartridges, and was separated on a C18 column; the calibration curve was linear over the range from 2 to 250 μg/kg of ceftiofur(m/z 524.22→m/z 125.34) in milk.

Ceftriaxone(ThamrongWongchang ET AL .2019)is a cephalosporin antibiotic drug used as first-line treatment for several bacterial diseases. Ceftriaxone belongs to the third generation ofantibiotics and is available as an intramuscular or intravenous injection.Previously published pharmacokinetic studies have usedhigh-performance liquid chromatography coupled with ultraviolet detection(HPLC-UV) for the quantification of ceftriaxone. This study aimed todevelop and validate a bioanalytical method for the quantification ofceftriaxone in human plasma using liquid chromatography followed bytandem mass spectrometry (LC-MS/MS). Preparation of sample wasperformed by protein precipitation withphospholipid-removal techniques for cleaning up matrix interferences. Thechromatographic separation was performed on an Agilent Zorbax EclipsePlus C18 column with 10 mM ammonium formate containing 2% formicacid: acetonitrile as mobile phase at a flow rate of 0.4 ml/min. Both theanalyte and cefotaxime (internal standard) were quantified using thepositive electrospray ionization (ESI) mode and selected reactionmonitoring (SRM) for the precursor-product ion transitions *m/z*555.0→396.1 for ceftriaxone and 456.0→324.0 for cefotaxime. At the concentration range of 1.01-200 μg/ml the method was validated . Linearity with correlation coefficient > 0.99 and no significant matrix effects were observed. The intra-assay and inter-assay precision were less than 5% and 10%, respectively and the acceptance criteria was ±15%.

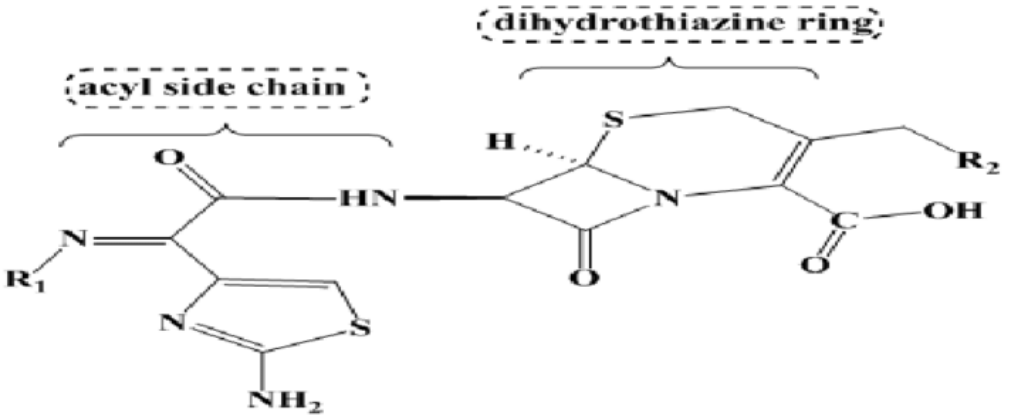
II. Conclusion

This review presented essential information regardingbioanalytical methods to determine the levels of 12 thirdgenerationcephalosporins using HPLC and LC-MS(/MS). We reviewed bioanalytical-related references covering the periodfrom 1987 to 2019, mostly focusing on recently developedbioanalytical methods for use in biological samples in animals and humans. The HPLC-UVmethod remains a powerful analytical tool with low instrumentcosts for third-generation cephalosporins. However, LC-MS(MS) provides improved specificity and sensitivity for measuringthird-generation cephalosporins with simple sample preparationprocedures and smaller sample volumes to inject, compared toHPLC-UV. Because certain cephalosporins, such as cefdinir, havestability issues during sample preparation, probably owing totheir chemical nature, full validation including stability tests onvarious storage conditions should be carefully considered priorto determination of cephalosporin levels in biological samples.

Further investigations with application of efficient LC-MS(/MS)to third-generation cephalosporins are required, as there havebeen few reports regarding third-generation cephalosporinsusing LC-MS(/MS) in biological samples.

Table 1: chemical structure and third generation cephalosporins

S



General structure of the cephalosporins(third generation)

Administration route	Drug name	pK_a	Salt form
Oral	cefdinir	1.3,3.3,9.7	Anhydrous
	Cefetamet pivoxil	8.21	-
	cefixime	2.10,3.73	Anhydrous
	Cefpodoxime proxetil	3.2	-
	ceftibuten	2.99,4.69	Dihydrate
parenteral	Cefmenoxime	3.04,4.14	Hydrochloride
	cefoperazone	2.55,9.55	Sodium
	cefotaxime	2.1,3.4,10.9	Sodium
	ceftazidime	1.8,2.7,4.1	Pentahydrate
	Ceftiofur	3.7	Sodium, Hydrochloride
	Ceftizoxime	2.95	Sodium
	Ceftriaxone	3,3.2,4.1	Disodium

Table 2: HPLC methods for individual determination of third generation cephalosporins in biological fluids

Cephalosporins	spicemen	Sample Pretreatment	column	Mobile phase	Detection
cefdinir	Human Plasma	Acid –based precipitation (perchloric acid,10%)	Diamonsil*C18(150×4.6mm,5.0μm)security guard cartridges (phenomenex* C18,4×2.0mm)	$NH_4H_2PO_4$(20mm,pH=3.0,adjusted by phosphoric:ACN(90:10 v/v)	UV,286nm
	Beagle dog plasma	Acid-based precipitation (perchloric acid,6% and online solid –phase extraction(SPE)	Trap column : lichroshewr C 18 (37×4.6mm,25μm) analytical column:L ultimate XB-C18(50×4.6mm,5μm)	Washing solvent:20 mm KH_2PO_4(pH- 3.0) Mobile phase: methanol : ACN: 20mm KH_2PO_4(1.25:6.75:82,v/v/v,pH - 3.0)	UV,286nm
	Human plasma and urine	Protein precipitation(ACN) And delipidation(methylene chloride)	Nova-pak C18 column	0.015m dibasic potassium phosphate:ACN(89:11,blister;88:12,plasma) adjusted to pH 3.3 for blister and 3.1 for plasma, with 85%phosphoric acid	UV,287nm
Cefetamet	Human plasma and urine	Protein precipitation(perchloric acid)	C18 reversed –phase column	Plasma:4mm $HCLO_4$:ACN(83:17,v/v)urine:4 mm$HCLO_4$: ACN(85:15,v/v)	UV,265nm
	Human plasma and urine	Plasma:protein precipitation(perchloric	Analytical column:	Plasma:4mm perchloric acid:ACN(83:17,v/v) Urine :4mm perchloric acid	

		acid)Urine:dilution(water)	spherisorb ODS (125×4mm,1.5µm) Hibar type (E.Merck)	:ACN(65:15,v/v)	UV,265nm
Cefixime	Serum and urine	Protein precipitation(MeOH)	TSK-LS410 ODS(150×4.6mm,5µm)	MeOH:0.03m phosphate buffer,pH-2.5(27:73,v/v)	UV,295nm
	Human plasma and urine	Protein precipitation(6% trichloro acetic acid)	RCM-100 Nov–pak C18(100×8mm,5µm)	Serum:170ml of ACN,1.36g of monobasic sodium phosphate,2ml of 85%phosphoric acid and 828ml of distilled water,pH 2.7 Urine: 200ml of ACN ,1.36g of monobasic sodium phosphate,2ml of 85% phosphoric acid and 798 ml of distilled water,pH 2.7	UV,280nm(serum)313nm(urine)
	Human plasma and urine	Protein precipitation(6% trichloro acetic acid)	RCM-100 Nova –pak C18(100×8mm,5µm)	Serum:170ml of ACN,1.36g of monobasic sodium phosphate,2ml of 85%phosphoric acid and 828ml of distilled water,pH 2.7 Urine: 200ml of ACN ,1.36g of monobasic sodium phosphate,2ml of 85% phosphoric acid and 798 ml of distilled water,pH 2.7	UV,280nm(serum)313nm(urine)
	Human serum and urine	Dilution of serum (1:10) and urine (1:10-100)samples in soerensen buffer with centrifugation	Nucleosil 5c18 (200×4mm,5µm)	MeOH: phosphate buffer (15:85v/v;43m mol dipotassium hydrogen phosphate and 1L water),pH 5.2 with phosphoric acid	UV,230nm
	Human plasma and urine	Plasma samples(1:2 to 1:100),dilu-ted with soerensen buffer and centrifuge	Nucleosil C18(200×4mm,5µm)	15% MeOH :sorenson's buffer (66.6 mm dipotassium hydrogen phosphate,PH 7.4 and 66.6mm potassium dihydrogen phosphate),pH 5.2 ACN:0.01 M phosphoric acid:0.1m monopotassium phosphate:water(13:201:66,v/v)	UV,230nm
	Human plasma and urine	Solid-liquid extraction (column switching)	Hitachi Gel (ODS)		UV,286nm
Cefmenoxime	Rat bile	Dilution with 0.1 M phosphate buffer (pH 7.0)	Nucleosil 5C18 column(150×4mm)	Water:ACN:acetic acid (50:10:1,v/v)	UV,254nm
	Human serum and urine	Protein precipitation(ACN) protein precipitation	C18 column	ACN:25mm acetic acid (32:69,v/v)	UV,254nm

	Human serum and urine	(per chloric acid) protein precipitation	µBondapak CN (300×3.9mm)	Acetate buffer(20mm) pH 3.8	UV,254nm
	Human serum		µBondapak CN (300×3.9mm)	ACN:0.05 M ammonium acetate (20:80,v/v)	UV,254nm
cefpodoxime	Human plasma and urine	Protein precipitation (12% perchloric acid)	C18 column	0.007 M Phosphoric acid : ACN (9:1,v/v)	UV,280nm
	Rabbit plasma	Protein precipitation	Analytical column: Cosmosil column,5C18-MS(150×4.6mm,5µm), pre column: phenomenex C18 (4×3mm) security guard cartridge	CH_3CN:10mm phosphate buffer, pH 3.5(30:70,v/v)	FL,430nm(excitation)and 556nm(emission)
	Human serum and urine	Protein precipitation(ACN), delipidation(dichloromethane)and dilution(10mM acetate buffer ,Ph 4)	Nucleosil C18(250×4mm,5µm)	Plasma:ACN:10mm acetate buffer,pH 4 (9:90,v/v) Urine:ACN:10mm acetate buffer,pH 4 (10:90,v/v)	UV,260nm
	human urine	solid-liquid extraction(column switching)	Phenomenex 1B-SIL C18	ACN:0.05m sodium acetate buffer (7:93,v/v)	UV,254nm
	human serum and urine	protein precipitation (ACN)and delipidation (dichloromethane)	Ultrasphere XL-ODS	ACN:21.5 mm ammonium acetate ,pH 5 (7:93,v/v)	
	human plasma	solid phase extraction	Phenomenex IB-SIL C18	ACN:MeOH:0.05m sodium acetate,ph 6(4.4:9.2,v/v)	UV,254nm UV,254nm
ceftibuten	Human plasma	Dilution (0.1% M ammonium acetate ,1:1,v/v)	Analytical column: waters µBondapak column (Phenyl packing in 300×4.6mm) Sample extraction and cleanup column:waters µBondapak (phenyl packing in a 150×3.9mm)	sample pre-column:0.1 m ammonium acetate (pH 6.51) Analytical column:2% ACN in o.1m ammonium acetate(pH 6.5)	UV,263nm
	Human plasma and urine	Dilution (0.2 M sodium phosphate buffer ,Ph 7.0)	Analytical column: waters µBondapak column (plasma)(300×3.9mm) Pre-column:waters µBondapak CN guard-PAK(urine)	Plasma: ACN and 0.05m ammonium acetate ,(2:98,v/v) Urine:ACN:0.05m sodium phosphate buffer pH 7 (2.5:97.5,v/v)	UV,254nm

cefoperazone	Human plasma and urine	plasma:protein precipitation (MeOH:0.1 M ammonium acetate (pH 5.2)2:1)	waters µBondapak C18 column (150×3.9mm) Lichrosorb	0.025 M ammonium acetate (pH 5.2):CH_3CN(84:16,v/v)	UV,245nm
	Rat bile	Urine:diluted (1:20 with mobile phase water,1:1) microdialysis	RP18column(250×4.6 mm,5µm)	100mm monosodium Phosphoric acid (pH 5.5):MeOH(70:30,V/V)	UV,254nm
	Human plasma	Protein precipitation (MeOH)	Hypersil® C18 column (250×4mm,5µm)	ACN:MeOH:5mm tetrabutyl ammonium hydroxide(13:9:78),pH 6.4	UV,230nm
	Human serum and muscle tissue	Protein precipitation (MeOH)	µBondapak phenyl(300×3.9mm)	ACN:0.005M tetrabutylammonium bromide(TBAB)buffer (20:80v/v)	UV,254nm
	Human serum and urine	Protein precipitation (MeOH-sodium acetate)	µBondapak C18 (300×3.9mm)	Gradient 1.2mm triethylamine:42mm acetic acid:ACN	UV,254nm
	Human plasma	Protein precipitation (MeOH)	µBondapak C18 (300×3.9mm)	ACN:MeOH:0.01 sodium acetate(15.2:0.8:84v/v/v)	UV,254nm
	Human serum		C18 column,5µm(250×4.6mm)	ACN:Tetramethylammonium chloride(TMAC):orthophosphoric acid :water(30:0.1:0.03:69.87,v/v)	UV,254nm
cefotaxime	Human serum	Protein precipitation(ACN)	µBondapak C18 (300×3.9mm)	ACN:0.01M acetate buffer,ph 4 (5:95 v/v)	UV,254nm
	Human serum	protein precipitation(trichloro acetic acid)	Lichrosorb RP-8(250×4.6mm,5µm)	MeOH:2Mm phosphoric acid (28:72,v/v)	UV,310nm
	Human serum and urine	protein precipitation (HCL)delipidation(chloroform-1-pentanol)and back extraction (phosphate buffer ,pH 7)	µBondapak C18 (300×3.9mm)	MeOH:0.01 M acetate buffer ph 4.8(15:85,v/v)	UV,234nm
	Human serum,urine,bile and saliva	protein precipitation(chloroform-acetone)	Spherisorb ODS (100×3mm)	MeOH:water:acetic acid(12:87:1,V/V)	UV,262nm
	Rat serum and bile	protein precipitation (perchloric acid) and dilution (sodium acetate)	Lichrosorb RP 18 (250×4mm,7µm)	ACN:MeOH:0.02M Phosphate buffer (10:7:83 v/v)	UV,254nm
		protein precipitation (phosphoric acid-MeOH)			

	Human serum	protein precipitation (ACN)	Lichrosorb RP 18 (250×4mm,7µm)	MeOH:PIC-A	UV,254nm
	Human serum	protein precipitation (2-propanol) and delipidation (chloroform-4% isoamyl alcohol)	Radial –pak c18(100×8mm)	MeOH:acetic acid(30:70,v/v),ph 5.5	
	human plasma nd urine	protein precipitation (ACN)and delipidation (ACN-1-butanol)	Radial - pak c18(100×8mm)	ACN : water (17:83,v/v)plus pic –A	UV,254nm
	Human plasma and urine	protein precipitation(MeOH)	µBondapak C18 (300×3.9 mm)	ACN:0.007 M phosphoric acid (15:85,v/v)	UV,270nm
	Rat plasma		RP-8, 10µM(250×4.6mm)	MeOH:0.02 M phosphate buffer, pH 4.5(23:77,v/v)	UV,254nm UV,245nm
Ceftazidime	Human plasma and urine	Protein precipitation (perchloric acid)	Hypersil ODS, 5µm	ACN:0.05M ammonium phosphate: formic acid (93:7:0.01,v/v)	UV,257nm
	Human serum and urine	Protein precipitation (MeOH)	µBondapak C18 (300×3.9mm)	MeOH:0.15m phosphate buffer ,pH 6.5(82:18,v/v)	UV,255nm
	Human plasma and urine	Protein precipitation(MeOH)	Micropak MCK 10 (300×4MM)	MeOH:50mm phosphate buffer (20:80,v/v),117µm perchloric acid	UV,257nm
	Human serum,urine,CSF and pdf	Protein precipitation (MeOH)	µBondapak C18 (300×3.9mm)	ACN:acetic acid:water(6:1:93,V/V)PH 4	UV,254nm
	Human and rabbit serum	Protein precipitation (MeOH-acetic acid)		ACN:acetic acid(10:90,v/v)	
	Human serum,urine and CSF	Protein precipitation (MeOH)	µBondapak C18 (300×3.9mm)	MeOH:01M sodium phosphate buffer (6:94,v/v)	UV,275nm
	Human serum	Column switching(C8 column)	Lichrosorb c18 (250×4.6mm,10µm) HP ODS (150×4MM,5µm)	ACN:10mm phosphate buffer ,ph 5(4:96,v/v)	UV,254nm UV,258nm
ceftizoxime	Human serum	Solid-liquid column	µBondapak C18 (300×3.9mm)	ACN:acetic acid (13:87,v/v)	UV,270nm
	Human serum	Protein precipitation(ACN) and delipidation	µBondapak C18	ACN:water:acetic acid(13:84:3,v/v)	UV,310nm

		(dichloromethane)	(300×3.9mm)		
	Human serum	Protein precipitation(perchloric acid)	Ultrasphere CN	MeOH:acetic acid (15:85,v/v)	UV,270nm
ceftriaxone	Human and dog plasma,urine and bile	Protein precipitation(ethanol)	Lichrosorb RP 18 (150×3.2 mm)	ACN:20mm phosphate buffer ph 7: tetra pentyl ammonium bromide (TPAB)(200:800:3.89,V/V/W)	UV,274nm
	Human plasma, urine and saliva	Protein precipitation(ACN)	Lichrosorb NH2 (250×4 mm)	ACN:water:ammonium carbonate (10%,w/v)	UV,274nm
	human plasma	protein precipitation(ACN) and Delipidation(dichloromethane)	Lichrosorb RP 8 (250×4 mm,5µm)	ACN:12.5mm phosphate buffer,PH 7:HDTMAB(40:60:2.73g,v/v/w)	UV,280nm
	human serum,urine and CSF	protein precipitation(MeOH)	ODS(250×4.6MM,10µm)	MeOH:phosphate buffer:tetrabutyl ammonium hydrogen sulfate(THBS)(20:80:1.75g,V/V/W)	ED,1.15V
		protein precipitation(ACN)	µBondapak C18(300×3.9mm)	ACN:10MM potassium phosphate,pH 9(46:54,v/v) Ion-pairing reagent : 10mm hexadecyl trimethylammonium bromide (HDTMAB)	UV,274nm

ACN-acetonitrile; ED- electrochemical detection ;FL- fluorescence;HDTMAB-hexadecyltrimethylammonium bromide;THBS-tetrabutyl ammonium hydrogen sulfate;TMAC-tetramethyl ammonium chloride;TPAB-tetrapentylammonium bromide;UV-ultraviolet

Table 3:HPLC methods for simultaneous determination of third generation in biological fluids

cephalosporins	specimen	Sample pretreatment	column	Mobile phase	detection
Cefdinir and cefixime	Human plasma	Protein denaturing and/or solvent extraction	Analytical column:supelco discovery HS C18 (150×4.6mm,5µm)pre-column cartridge:perkin elmer RP 18(300×4.6MM,10µM)	ACN:MeOH(50:50v/v):0.05% TFA solution (aqueous)(19:81,v/v)	UV,285nm
Cefixime,cefaclor,cefadroxil,cephalexin and cephradin	Human serum	Serum protein precipitation with 0.1ml of ACN	Analytical column:atlex ultrasphere –octy(c8) column (150×4.6mm,5µm) Pre column:waters RCSS silica guard pak pre-column	MeOH:monobasic sodium phosphate buffer ,125mmol/L(20:80),v/v), pH 2.6 with concentrated phosphoric acid	UV,240nm
Cefotaxime, cefoperazone , cefmenoxime, ceftazidime and ceftriaxone	Human serum ,urine and bile	Protein precipitation and dilution	High-speed analytical column(75×4.6mm)filled with 3µm diameter octadecyl silane-coated silica particles	A mixture of 20mm ammonium acetate and ACN adjusted to pH5 with filtered glacial acetic acid	UV,254nm
Cefaperazone,cefixime ,ceftazidime,ceftizoxime,ceftriaxone and cefotaxime (cefepime)	Human plasma and amniotic fluid	SPE	Analytical column:Xterra C18 column(250×4.6mm,5µm) Pre-column:phenomenex C18 guard column (4×3.0mm)	Gradient elution:0-5 min (18% MeOH 18-45%)15-16min (MeOH 45-55%),16-21 min,21-22 min(MeOH 55-18%)	UV,285nm (cefixime,ceftizoxime),260nm(cefoperazone)
Cefoperazone,ceftiofur	milk	SPE	Inertsil ODS-3	Gradient elution	DAD,cefotax

and cefotaxime			(250×4mm,5µm)	A:$CH_3COONH4$0.05M AND B :ACN:0-12 min (93:7,v/v),12-18 min(85:15,v/v)18-23min (65:35,v/v)	ime and ceftiofur at 265nm,cefoperazone at 275nm
Cefotaxime and ceftizoxime	Human serum,urine,blister,fluid	Protein precipitation (ACN) followed by delipidation (methylene chloride)	Nova-pak C18(4µm)	0.2m sodium acetate and 0.2m acetic acid in water:MeOH(80:20)	UV,254nm
Cefotaxime,ceftazidime and ceftriaxone	Human plasma	SPE	Analytical column:atlantic T3 (150×4.6mm,5µm) Pre-column:atlantis T3 guard column(20×4.6mm,5µm)	10mm phosphoric acid solution,adjusted to ph 2 with HCl and ACN, a linear gradient from 7 to19% ACN in 6 min and from 19 to 49% from 6 to 16 min was used with a flow rate of 2ml/min.run time was prolonged to 22 min to return to intial conditions	UV,230nm
Ceftizoxime and cefazolin	Human serum	-	Kromasil 100,C18 (250×4.6mm,5µm)	ACN:water(60:40;v/v)	UV,270nm
Ceftazidime,cefotaxime and ceftriaxone	Human serum	Protein precipitation(ACN) and removal of lipid soluble components (chloroform)	waters X-bridge C18 column(30×4.6mm,2.5µm silica)	1A:ACN:50mm phosphate buffer PH 2.4(8:92) 1B:ACN:50MM phosphate buffer pH2.4(12:88)	UV,260nm
Ceftriaxone,metronidazole and levofloxacin	Human urine	Protein precipitation(ACN)	Kromasil 100,C18(250×4.6MM,5µM)	1.5mm KH_2PO_4 (PH 4.5 WITH PHosphoric acid)and 0.0125% triethylamine:MeOH(70:30,v/v)	UV,247nm
Ceftibuten,cefixime,cefaclor and cefuroxime	Human plasma	-	-	-	-

MeOH-methanol; TFA- Trifluoroacetic acid; UV- ultra violet

Table 4: LC-MS/MS methods for determination of third generation cephalosporins in biological fluids

cephalosporins	spicemen	Sample pretreatment	column	Mobile phase	System and mass spectrometric detection
cefdinir	Rat plasma,urine	Protein precipitation(MeOH)	Synergi 4µ polar –RP 80A column(150×2.0mm,4µm)	Isocratic elution consists of 0.1%formic acid and MeOH (65:35,v/v)at a flow rate of 0.2ml/min	HPLC-MS/MS in positive mode m/z 396.1→m/z 227.2
	Human plasma	Protein precipitation(10 TCA aqueous solution)	rp18 waters symmetry shield column((50×2.1mm,5µm)	Isocratic elution consists of ethanol:water:formic acid(25:75:0.075,v/v/v) at a flow rate of 0.2ml/min	HPLC-MS/MS by SRM in positive mode m/z 396.1→m/z 226.9
cefetamet	Human plasma	Protein precipitation(ACN)	RP eclipse XDB C18 (100×2.1mm,3.5µm)	Isocratic elution consists of 0.1% formic acid:CAN(45:55,v/v)at a flow rate of 0.3ml/min	HPLC-MS/MS in positive mode m/z 398.1
	Human plasma	Protein precipitation(ACN)	Zorbax SB C8 column(150×4.6mm,5µm)	Isocratic elution consists of ACN:water:formic acid(40:60:0.5,v/v/v)at a flowrate of 0.5ml/min	HPLC-MS/MS by SRM in positive mode m/z 454→m/z 285
	Human plasma	SPE(supeldean LC_18 SPE tube)	Diamonsil C18 column(250×4.6mm,5µm)	Isocratic elution of MeOH:water:formic acid (80:20:1,v/v),delivered at aa flow rate of 0.55ml/min	HPLC-MS/MS by SRM in positive mode m/z 398→m/z 241
cefixime	Human plasma	Protein precipitation(ACN)	Zorbax eclipse XBD C18 column(150×4.6mm,5µm)	Isocratic elution consists of ACN:MeOH:0.5%formic acid(23:10:67,v/v)at a flow rate of 0.6ml/min	HPLC-MS/MS in positive mode m/z 453.8
	Human plasma	Protein			

		precipitation(ACN)	Zorbax SB C8 column(150×4.6mm,5 µm)	Isocratic elution consists of ACN:water:formic acid(40:60:0.5,v/v/v)at a flow rate of 0.5ml/min	HPLC-MS/MS by SRM in positive mode m/z 454→m/z 285
cefpodoxime	Human plasma	SPE(sample preparation,C18 cartridges)	Princeton SPHER C18 column(150×4mm,5µm)	Isocratic elution using MeOH :CAN : ammonium acetate 2mm,ph 3.5)(25:25:50,v/v/v)flow rate of 0.8sml/min	HPLC-APCI-MS by SIM in negative mode m/z 408
ceftibuten	Human sputum,plasma	Direct external standard acid-based precipitation(0.1 m ammonium acetate solution)	Waters µbondapak phenyl column(300×4.6mm)	Isocratic elution consists of 2% CAN in 0.1mm= ammonium acetate at a flow rate of 1.0ml/min	LC-LC-TSP-MS in positive mode m/z 226
cefoperazone	Human plasma	LLE(ethyl acetate)	Waters Xterra C18 column(50×2.1mm,5µm)	Isocratic elution consists of MeOH:ammonium formate solution(10mm,pH 4.5)(30:70,v/v)at a flow rate of 0.2ml/min	HPLC-MS/MS by SRM in negative mode m/z 644.1→m/z 528.0
	Human plasma	Protein precipitation(ACN)	Mightysil RP18 column(150×2.0mm,5 µm)	Gradient elution of MeOH in 0.1% formic acid with a flow rate of 0.2ml/min	HPLC-MS by SIM in positive mode m/z 646
	Bovine milk	LLE(potassium oxalate sodium hydrogen phosphate solution,leadacetate solution),and SPE (oasis HLB cartridges)	Waters acquity UPLC BEH C18 column(50×2.1mm,1.7 µm)	Gradient elution consists of mobile phase A (water containing 0.1% formic acid)and mobile phase B (ACN containing 0.1% formic acid)at a flow rate of 0.25ml/min	UPLC-MS/MS in negative mode or positive mode m/z 644.0→m/z 115.0
	Milk	SPE(C18-Fe3O4@mSio2 microsphere)eluting solvents (ACN,chloroform,MeOH,acetone or ethyl acetate)	Ultimate XB—C18 column(100×2.1mm,5 µm)	Gradient elution consists of mobile phase A (water)and mobile phase B (CAN)both acidified with 0.1% formic acid with a flow rate of 0.3ml/min	HPLC-MS/MS by MRM in positive mode m/z 530
	Cow milk	Defat(ACN) and SPE(oasis HLB cartridges or strata X cartridge)	Zorbax eclipse XDB-C8 column(150×4.6mm,5 µm)	Gradient elution using water and MeCN with 0.1% formic acid at a flow rate of 1ml/min ,the intial mobile phase was composed of H20:MeCN (85:15,v/v) with a PH of 3.2 Gradient elution using water and MeCN with 0.1% formic acid at a flow rate of 1ml/min ,the intial mobile phase was composed of H20:MeCN (88:12,v/v)	HPLC-MS/MS by MRM in positive mode m/z 646→m/z 290
			Waters UPLC BEH shield RP18 (50×2.1mm,1.7µm)	Gradient elution using mobile phase A(0.1% formic acid in water) and mobile phase B (MeOH)at a flow rate of 0.30ml/min	UPLC-MS/MS by MRM in positive mode m/z 640→m/z 290
	milk	SPE	Acquity BEH shield RP 18 column (100×2.1mm,1.7µm)		UPLC-MS/MS in positive mode m/z 646.45→m/z 143.05
Cefotaxime	Milk	SPE	Acquity BEH shield RP 18 column (100×2.1mm,1.7µm) Waters acquity UPLC	Gradient elution using mobile phase A(0.1% formic acid in water) and mobile phase B (MeOH)at a flow rate of 0.30ml/min	UPLC-MS/MS in positive mode m/z 396.16

	Bovine milk	LLE (potassium oxalate sodium hydrogen phosphate solution,leadacetate solution),and SPE(oasis HLB cartridges)	BEH C18 column(50×2.1mm,1.7 µm)	Gradient elution using mobile phase A (water containing 0.1% formic acid)and mobile phase B (CAN containing 0.1% formic acid)at a flow rate of 0.25ml/min	UPLC-MS/MS in negative mode or positive mode m/z 454.0→m/z 239.0
ceftazidime	Human plasma	SPE(oasis MCX µ-elution 96-well plates)	Acquity HSS T3 column(50×2.1mm,1.7 µm)	Gradient elution consists of 1mm CH_3COOH/CH_3COONH_4-buffer with 5% ACN and ACN at a flow rate of 0.6ml/min	UPLC-MS/MS by MRM in positive mode m/z 547.1
	Human plasma	Protein precipitation(ACN) and LLE (CAN and dichloromethane)	Acquity UPLC BEH C18 column(100×2.1mm,1.7µm)	Gradient elution of water and ACN,both containing 0.1% formic acid,at a flow rate of 0.4ml/min	UPLC-MS/MS by MRM in positive mode m/z 547.22→m/z 468.10
	Human plasma	Protein precipitation(ACN cointaining 0.1 formic acid))	RP kinetex C18 column(50×2.1mm,2.6 µm)	Gradient elution mobile phase A(water containing 0.1% formic acid)and mobile phase B(ACN containing 0.1% formic acid)at a flowrate of 0.3ml/min	HPLC-MS/MS by MRM in positive mode m/z 547.2→m/z 167.1
ceftiofur	Cow milk	Defat (ACSN) and SPE(oasis HLB cartridges or strata X cartridge)	Zorbax eclipse XDB-C8 column(150×4.6mm,5 µm)	Gradient elution consists of water and MeCN with 0.1% formic acid .the initial mobile phase was H2O:MECN(85:`15,v/v)with a Ph OF 3.2 at a flow rate of 1ml/min	HPLC-MS/MS by MRM in positive mode m/z 524→m/z285
			Waters UPLC BEH shield RP18 (50×2.1mm,1.7µm)	Gradient elution consists of water and CAN with 0.1% formic acid .the initial mobile phase was H2O:CAN (88:12,v/v)at a flow rate of 1ml/min	UPLC-MS/MS by MRM in positive mode m/z524→m/z285
	Milk	Protein precipitation(ACN)	Altima C18 column(150×2.1mm,5 µm)	Gradient elution using mobile phase A (water) and mobile phase B(CAN),each containing 0.1%formic acid at a flow rate of 0.25ml/min	HPLC-MS/MS in positive mode m/z 524→m/z 241
	milk	SPE	Acquity BEH shield RP18 column(100×2.1mm,1.7µm)	Gradient elution using mobile phase A (0.1% formic acid in water)and mobile phase b (meoh)at a flow rate of 0.30ml/min	UPLC-MS/MS in positive mode m/z524.22→m/z 125.34

References:

[1]. Ahmed S,Abbel-Wadood HM and Mohamed NA.Highly sensitive and selective high-performance liquid chromatography method for bioequivalence study of cefpodoxime proxetil in rabbit plasma via fluorescence labeling of its active metabolite.Journal pf Chromatography B: Analytical Technology in Biomedicine and Life Sciences 2013: 34-40; doi: S1570-0232(13)00357-7 [pii]10.1016/j.jchromb.2013. 06.036.

[2]. Alekseev VG.Acid-base properties of penicillins and cephalosporins(areview).Pharmaceutical chemistry journal 2010;44(1): 14-24.

[3]. Anacona JR, Bravo A and Lopez ME. Cefoperazone metal complexes:synthesis and characterization. Journal of the Chilean Chemical Society2013; 58(1): 1520–1523.

[4]. Arayne MS, Sultana N and Bi Bi Z. Simultaneous determination of cefazolin or ceftiozoxime in presence of vitamin C from pharmaceutical formulation and human serum by RP-HPLC. Pakistan Journal of Pharmaceutical Sciences 2007a; 20(1): 56–61.

[5]. Ayrton J. Assay of ceftazidime in biological fluids using high-pressure liquid chromatography. Journal of Antimicrobial Chemotherapy 1981; 8(suppl. B.): 227–231.

[6]. Barriere SL and Flaherty JF. 3rd-Generation cephalosporins – a criticalevaluation.Clinical Pharmacy 1984; 3(4): 351–373.

[7]. Bergan T and Solberg R. Assay of cefotaxime by high-pressure–liquidchromatography. Chemotherapy 1981; 27(3): 155–165.

[8]. Bombardt PA, Cathcart KS, Bothwell BE and Closson SK. Determination ofcefpodoxime levels and cefpodoxime stability in human urine by directinjection HPLC with column-switching. Journal of Liquid Chromatography1991; 14(9): 1729–1746; doi: 10.1080/01483919108049650.

[9]. Bompadre S, Ferrante L, Alò FP and Leone L. On-line solid-phase extractionof ceftazidime in serum and determination by high-performanceliquid chromatography. Journal of Chromatography B: Biomedical Sciencesand Applications 1995; 669(2): 265–269; doi: 10.1016/0378-4347(95)00100-W.

[10]. Borner K, Borner E, Lode H and Peters A. Determination of cefmenoximein human body fluids by high-performance liquid chromatography.European Journal of Clinical Microbiology 1983; 2(1): 17–21.

[11]. Borner K, Hartwig H, Lode H and Saathoff N. Liquid chromatographic determinationof cefpodoxime in human serum and urine. Fresenius'Journal of Analytical Chemistry 1990; 337(1): 124.

[12]. Bowman DB, Aravind MK, Miceli JN and Kauffman RE. Reversed-phase high-performance liquid chromatographic method to determine ceftriaxone in biological fluids. Journal of Chromatography 1984; 309(1):209–213.

[13]. Brisson AM and Fourtillan JB. Determination of cephalosporins in biological material by reversed-phase liquid column chromatography. Journal of Chromatography 1981; 223(2): 393–399.

[14]. Carlier M, Stove V, Roberts JA, Van de Velde E, De Waele JJ and Verstraete AG. Quantification of seven beta-lactam antibiotics and two betalactamase inhibitors in human plasma using a validated UPLCMS/ MS method. International Journal of Antimicrobial Agents 2012; 40(5): 416–422; doi: S0924-8579(12)00277-4 [pii]10.1016/j.ijantimicag.2012.06.022.

[15]. Chang YL, Chiou SH, Chou YC, Yen CJ and Tsai TH. Quantitative determination of unbound cefoperazone in rat bile using microdialysis and liquid chromatography. Journal of Pharmaceutical and Biomedical Analysis 2007; 45(1): 158–163; doi: S0731-7085(07)00262-2 [pii]10.1016/j.jpba.2007.05.002.

[16]. Chen J, Jiang B, Lou H, Yu L and Ruan Z. Bioequivalence evaluation of cefdinir in healthy fasting subjects. Arzneimittel-Forschung 2012;62(1): 9–13; doi: 10.1055/s-0031-1291361.

[17]. Chen X, Zhong D, Huang B and Cui J. Determination of cefaclor in human plasma by a sensitive and specific liquid chromatographic–tandem mass spectrometric method. Journal of Chromatography B: Analytical Technology in Biomedicine and Life Sciences 2003; 784(1): 17–24; doi:S1570023202006566 [pii].

[18]. Chen ZJ, Zhang J, Yu JC, Cao GY, Wu XJ and Shi YG. Selective method for the determination of cefdinir in human plasma using liquid chromatography electrospray ionization tandam mass spectrometry. Journal of Chromatography B: Analytical Technology in Biomedicine and Life Sciences 2006; 834(1–2): 163–169; doi: S1570-0232(06)00165-6 [pii] 10.1016/j.jchromb.2006.02.047.

[19]. Colin P, De Bock L, T'Jollyn H, Boussery K and Van Bocxlaer J. Development and validation of a fast and uniform approach to quantifybeta-lactam antibiotics in human plasma by solid phase extraction– liquid chromatography–electrospray–tandem mass spectrometry.Talanta 2013; 103: 285–293; doi: S0039-9140(12)00877-6 [pii] 10.1016/j.talanta.2012.10.046.

[20]. Daeseleire E, De Ruyck H and Van Renterghem R. Confirmatory assay for the simultaneous detection of penicillins and cephalosporins in milk using liquid chromatography/tandem mass spectrometry. RapidCommunications in Mass Spectrometry 2000; 14(15): 1404–1409; doi:10.1002/1097-0231(20000815)14:15<1404::AID-RCM38>3.0.CO;2-4.

[21]. Danzer LA. Liquid-chromatographic determination of cephalosporins and chloramphenicol in serum. Clinical Chemistry 1983; 29(5): 856–858.

[22]. Dell D, Chamberlain J and Coppin F. Determination of cefotaxime and desacetylcefotaxime in plasma and urine by high-performance liquid chromatography. Journal of Chromatography 1981; 226(2): 431–440.

[23]. Demotes-Mainard FM, Vinçon GA, Jarry CH and Albin HC. Micromethod for the determination of cefotaxime and desacetylcefotaxime in plasma and urine by high-performance liquid chromatography. Journal of Chromatography B: Biomedical Sciences and Applications 1984; 336(1): 438–445; doi: 10.1016/S0378-4347(00)85174-9.

[24]. Dhib M, Moulin B, Leroy A, Hameau B, Godin M, Johannides R and Fillastre JP. Relationship between renal function and disposition oforal cefixime. European Journal of Clinical Pharmacology 1991; 41(6):579–583; doi: 10.1007/BF00314988.

[25]. Dokladalova J, Quercia GT and Stankewich JP. High-performance liquidchromatographic determination of cefoperazone in human serumand urine. Journal of Chromatography 1983; 276(1): 129–137.

[26]. Dubala A, Nagarajan JS, Vimal CS and George R. Simultaneous quantificationof cefpodoxime proxetil and clavulanic acid in human plasma byLC-MS using solid phase extraction with application to pharmacokineticstudies. Journal of Chromatography B: Analytical Technology inBiomedicine and Life Sciences 2013; 921–922: 49–55; doi: S1570-0232(13)00060-3 [pii]10.1016/j.jchromb.2013.01.018.

[27]. Duverne C, Bouten A, Deslandes A, Westphal JF, Trouvin JH, Farinotti Rand Carbon C. Modification of cefixime bioavailability by nifedipinein humans – involvement of the dipeptide carrier system. AntimicrobialAgents and Chemotherapy 1992; 36(11): 2462–2467.

[28]. Fabre H, Eddine NH, Berge G and Blanchin MD. Overlapping ionizationconstantsfor cefotaxime. Journal of Pharmaceutical Science 1985;74(1): 85–86; doi: 10.1002/jps.2600740123.

[29]. Falkowski AJ, Look ZM, Noguchi H and Silber BM. Determination ofcefixime in biological samples by reversed-phase high-performanceliquid chromatography. Journal of Chromatography 1987; 422: 145–152.

[30]. Fasching CE, Peterson LR and Gerding DN. High pressure liquid chromatographicanalysis for quantitation of BMY-28142 and ceftazidimein human and rabbit serum. Journal of Liquid Chromatography 1986;9(8): 1803–1814.

[31]. Faulkner RD, Bohaychuk W, Desjardins RE, Look ZM, Haynes JD, Weiss AIand Silber BM. Pharmacokinetics of cefixime after once-a-day andtwice-a-day dosing to steady state. Journal of Clinical Pharmacology1987; 27(10): 807–812.

[32]. Fubara JO and Notari RE. A kinetic oxymoron: concentration-dependentfirst order rate constants for hydrolysis of ceftazidime. Journal ofPharmaceutical Science 1998; 87(1): 53–58; doi: 10.1021/Js970179k.

[33]. Granich GG and Krogstad DJ. Ion pair high-performance liquid chromatographicassay for ceftriaxone. Antimicrobial Agents and Chemotherapy1987; 31(3): 385–388.

[34]. Hakim L, Bourne DW and Triggs EJ. High-performance liquid chromatographicassay of cefotaxime, desacetylcefotaxime and ceftriaxone inrat plasma. Journal of Chromatography 1988; 424(1): 111–117.

[35]. Hamacher J, Luepke J, Reidenberg BE, Nord CE, Borner K, Koeppe P,Bristol D and Lode H. Changes in fecal flora and comparativemultiple-dose pharmacokinetics of ceftibuten, cefpodoxime proxetiland amoxycillin/clavulanate. Clinical Microbiological Infection 1999;5(6): 339–354.

[36]. Hamashima Y, Kubota T,Minami K, Ishikura K, Konoike T, YoshiokaM, YoshidaT, Nakashimizu H and Motokawa K. Synthesis and biological propertiesof 7 beta-[(Z)-2-(2-amino-4-thiazolyl)-4-carboxy-2-butenoylamino]-3-cephem-4-carboxylic acid (7432-S), a new oral cephem antibiotic.Journal of Antibiotics (Tokyo) 1987; 40(10): 1468–1470.

[37]. Holt DE, De Louvois J, Hurley R and Harvey D. A high performance liquidchromatography system for the simultaneous assay of some antibioticscommonly found in combination in clinical samples. Journal ofAntimicrobial Chemotherapy 1990; 26(1): 107–115.