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HPLC ANALYSIS OF CEPHALOSPORINS AND STUDY OF DIFFERENT ANALYTICAL PARAMETERS

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ABSTRACT

Cephalosporins are the most important type of antibiotics used vary widely. Analysis of these antibiotics is challenge because of their sensitivity and instability to different conditions. The present review is extended to find out different HPLC methods used for analysis for cephalosporins in formulations and biological fluids. The chromatographic conditions used for the analysis as well as analytical parameters study carried out with different experimental conditions and different combinations. During the study analytical parameters studied such as range, linearity, precision, accuracy, LOD, LOQ etc. The focus of study of analysis of cephalosporin in formulation and biological fluid are useful for the determination of various cephalosporin for budding researchers.

INTRODUCTION: Although there are several classification schemes for antibiotics, based on bacterial spectrum (broad, narrow) or route of administration (injectable, oral, topical), or type of activity (bactericidal, bacteriostatic), the most useful is based on chemical structure. Antibiotics within a structural class will generally have similar patterns of effectiveness, toxicity, and allergic potential.

Most commonly used type of antibiotics are Pencillins, Fluoroquinolones, Cephalosphorins, Macrolides and Tetracyclines. While each class is composed of multiple drugs, each drug is unique in some way. Antibiotics are obtained from three major sources such as, microorganisms, synthesis and semi synthesis.

1. **Microorganisms:** For example, bacitracin and polymyxin are obtained from some Bacillus species streptomycin tetracycline etc. from streptomyces species; gentamycin from *Micromonospora purpura*; griseofulvin and some penicillin and cephalosporin from certain genera (penicillium, acremoni) of the family Aspergillacea; and monobactam from *Pseudomonas acidophila* and Gluconobacter species. Most antibiotics in current use have been produced from streptomyces species.
2. **Synthetic:** Chloramphenicol is now produced by a synthetic process.
3. **Semi-synthesis:-** This mean that part of the molecule is produced by a fermentation process using the appropriate microorganism and the products is then further modified by a chemical process. Many penicillin and cephalosporin are produced in this way.

Cephalosporins:

History: Antibiotics are the most important bioactive and chemotherapeutic compounds made by microbiological synthesis. They also include antimicrobial compounds present in higher plants and animals. They have proven their significance in varied fields like medicinal chemistry, agriculture and food industry.

Up to now about 40000 antibiotics have been found and about 80 of them are in therapeutic use. They are isolated primarily from metabolic products of living cells. Various penicillins, cephalosporins and several other antibiotics are semi-synthetic ones, which mean one part of the molecule, i.e. 6-amino penicillanic acid is prepared from say penicillin G or penicillin V, followed by synthetic introduction of an appropriate side chain [1].

Cephalosporin compounds were first isolated from cultures of *Cephalosporium acremonium* from a sewer in Sardinia in 1948 by Italian scientist Giuseppe Brotzu. He noticed that these cultures produced substances that were effective against *Salmonella typhi*, the cause of typhoid fever, which had beta-lactamase. Guy Newton and Edward Abraham at the Sir William Dunn School of Pathology at the University of Oxford isolated cephalosporin C.

Cephalosporins are structurally and pharmacologically related to the penicillins. Like the penicillins, cephalosporins have a β-lactam ring structure that interferes with the synthesis of the bacterial cell wall. They are used for the treatment of infections caused by Gram (+) and Gram (−) bacteria. They are among the safest and the most effective broad-spectrum bactericidal antimicrobial agents and therefore, they are the most frequently prescribed class of antibiotics [2].

To date, more than 20 cephalosporins have been approved by the U.S. Food and Drug Administration, and new ones continue to appear. Cephalosporins have an excellent safety profile and a basic structure that lends itself to numerous alternations, resulting in compounds that have widely different spectra of antimicrobial activity. The continuing emergence of resistant strains of bacteria necessitates the development of new antibiotics.

Structure: Cephalosporins like penicillins having a b-lactam ring antibiotic containing a dihydrothiazine ring with D-a-aminoadipic acid. Cephalosporins are less toxic and broad-spectrum antibiotics comparable in action to ampicillin. Organisms known to produce cephalosporin C include *Acremonium chrysogenum* and *Acremonium chrysogenum* ATCC 36225 [3, 4].

The cephalosporin ring structure is derived from 7-aminocephalosporanic acid (7-ACA) while the penicillins are derived from 6-aminopenicillanic acid (6-APA). The first cephalosporin, known as cephalosporin c, was isolated from the fermentation products of a fungus, *Cephalosporium acremonium* from a sewer in Sardinia in 1948 by Italian scientist Giuseppe Brotzu. He noticed that these cultures produced substances that were effective against Salmonella typhi, the cause of typhoid fever Hydrolysis of this compound produced aminocephalosporanic acid, which was modified with different side chains to create the whole family of cephalosporin antibiotics.

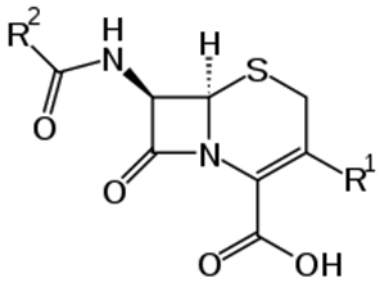
The cephalosporin nucleus, 7-aminocephalosporanic acid (7-ACA), was derived from cephalosporin C and proved to be analogous to the penicillin nucleus 6-aminopenicillanic acid, but it was not sufficiently potent for clinical use. Modification of the 7-ACA side-chains resulted in the development of useful antibiotic agents, and the first agent cephalothin (cefalotin) was launched by Eli Lilly in 1964.

The cephalosporin molecule is composed of a nucleus and two side chains. The nucleus 7-aminocephalosporinic acid consists of a four member's β-lactam ring fused to a six-member dihydrothiazine ring. By comparison, the penicillin nucleus, 7-aminopenicillanic acid has β-lactam ring fused to a five-member thiazolidine ring.

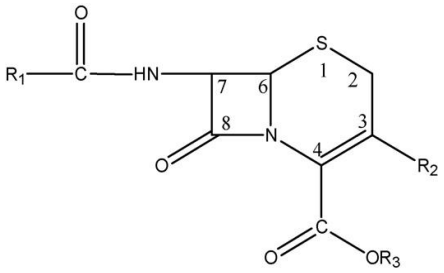
The cephalosporin nucleus has two advantages over the penicillin nucleus. First, it is inherently more stable to β-lactamases; bacteria that produce penicillinase are usually susceptible to cephalosporin. The second advantage is the presence of R2 substituent. The penicillin molecule can be modified at only one site (R1), while the cephalosporin molecule has two sites (R1 and R2). Thus, compared with penicillins the potential number of cephalosporins is considerably greater.

The presence of an intact β-lactam ring is essential for the antimicrobial activity of the cephalosporin. Modifications of the R1 substituent, which is in close proximity to the β-lactam ring, affect antibacterial activity by altering β-lactamase stability and/or binding affinity for the penicillin-binding proteins.

Changes in the R2 which is attached to the dihydrothiazine ring, affect the pharmacokinetic property of the drug. Substitution of an acetoxy group ([-CH.sub.2]-O-CO [-CH.sub.3]) for R2 is associated with significant metabolism to desacetyl derivatives. The introduction of a methoxy group (-O[CH.sub.3]) on the β-lactam ring at position 7 is associated with a marked increase in both B-lactamase stability and activity against anaerobes.

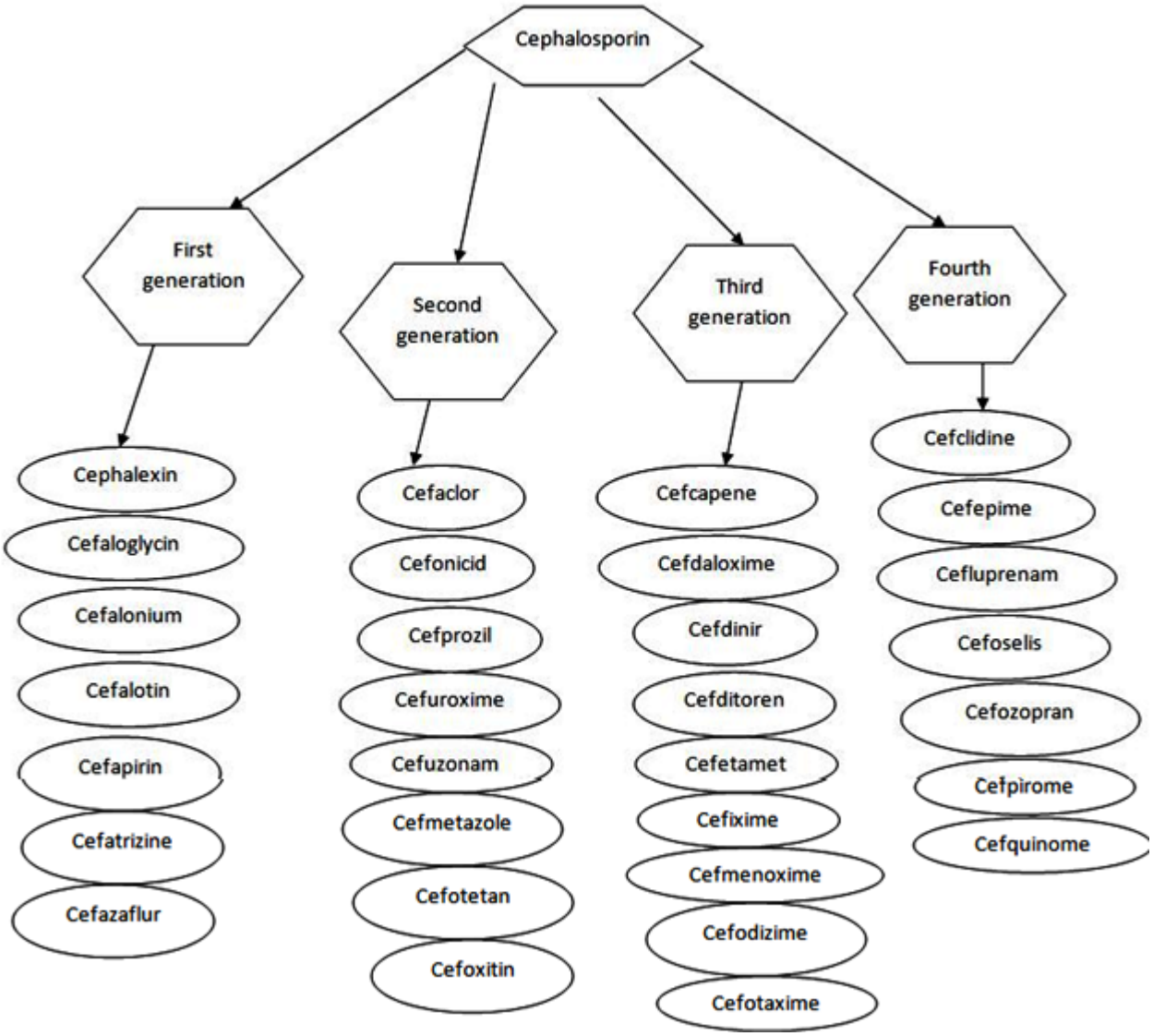


CORE STRUCTURE OF CEPHALOSPORIN



STRUCTURE OF CEPHALOSPORIN

Classification of Cephalosporins:



Analysis of Cephalosporin: There are various methods used for the analysis of cephalosporin in the various forms like chromatographic, UV, electrophoresis etc. The applications of HPLC 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 and to improve 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 HPLC has ability to analyze both volatile and nonvolatile compounds with ultra trace level may be employed in clinical research. Many antibiotics contain ionizable group can be analyzed by ion exchange chromatographic methods. High resolving power of HPLC serves as a particularly important method for isolation and purification of antibiotics [5].

Official Methods of Analysis: The *United States Pharmacopeia XXX* [6] prescribes a polarographic method for the assay of cefamandole naftate and HPLC methods for the assay of the other cited cephalosporins while the *European Pharmacopeia 2002* [7] prescribes HPLC methods for their assay. The analysis of cephalosporins in biological materials from human origin and in food-producing animals [8] foods, waters and pharmaceuticals was performed with liquid chromatographic, capillary electrophoretic, spectroscopic and electrochemical methods. None of them, however, was analyzed the seven cephalosporins simultaneously [9, 10].

Analysis in Bulk Drug and Dosage Forms: There are various methods available for the analysis of antibiotics in different formulations as well as in biological fluids. Some methods found in literature survey are for single cephalosporin while some methods are available with different combination. Various methods found are on different instrumental methods such as, HPLC, HPTLC, GC, CE, TLC, UV spectrophotometric, AAS, Electrochemical methods.

Chromatographic methods:

High-Performance Liquid Chromatographic Methods (HPLC): The most common methods for the analysis of cephalosporins in formulation and in biological fluids are HPLC method for analysis. As reported in the literature, several analytical procedures have been described for analysis of cefotaxime, ceftazidime and ceftriaxone in pharmaceutical formulations and biological fluids. These include high-performance thin layer chromatography [11], high-performance liquid chromatography [12-14], differential pulse adsorptive stripping voltammetry [9], NMR spectrometry [15], polarography [16] and UV derivative spectrophotometry [17].

There are various HPLC methods are reported for the analysis of a single cephalosporin in biological fluid [18-25]. All these methods present a unique preparatory and chromatographic protocol for single cephalosporin or its metabolite or both of them. Several methods have been used for the analysis of Ceftazidime the third generation cephalosporin for estimation of ceftazidime alone which includes High Performance Liquid Chromatography [26-28] and in combination with pyridine [29] Vancomycin [30] and cefepime [31].

The impressive increase in the use of high-performance liquid chromatography in the past thirty years did not pass the b-lactam antibiotics. HPLC has been used frequently in all fields of B-lactam research, not only as an assay method but also as a tool for purification of the antibiotics. Due to the insolubility of these compounds in organic solvents, normal phase LC was sparingly used. Most methods employ reversed-phase or ion-pair reversed-phase LC and chemically bonded packing materials.

A review on the evidence of chemical structure and stability profiles of ceftazidime [17] and cefotaxime [32] has been reported. Recently, full spectrum quantitation (FSQ) was used for rapid multi-component analysis of complex biological and pharmaceutical mixtures [33]. The technique was successfully applied for simultaneous analysis of binary mixtures of cortisonecortisol [34], cephalexinxephradine, phenobarbiton ephenytoin sodium and ternary mixture of aspirin-caffeine salicylic acid [35].

Moreover, FSQ has been used to evaluate the stability of chloramphenicol succinate in injections [36] and to determine famotidine [37] in the presence of its acid induced degradation products. The present work reports on the use of FSQ and HPLC to quantify cefotaxime, ceftazidime and ceftriaxone in the presence of their alkali-induced degradation products and in commercial injections. Furthermore, the application of HPLC as a stability-indicating assay to study the kinetics of degradation of the investigated antibiotics in aqueous solutions of varying pH (2-10) is demonstrated.

Spectroscopic methods:

Ultraviolet Spectrophotometric Methods: Cefotaxime, ceftriaxone and ceftazidime were determined in the presence of their alkali-induced degradation products through spectrophotometric full spectrum quantitation over the range of 265-230 nm [37]. Various UV spectrophotometric methods are reported for the analysis of Ceftazidime alone in presence of other drugs [38-42].

Mixtures of ceftazidime, cefuroxime sodium, cefotaxime sodium and their degradation products were analyzed by first-derivative spectrophotometry at 268.6, 306, 228.6 nm, respectively. Cefotaxime and cefuroxime were determined through the reaction with 1 chlorobenzotriazole and the absorbance was measured at 298 nm.

UV, first-derivative, second-derivative and H-point standard addition methods were applied for the determination of cefalexin in pharmaceutical preparations. Derivative spectrophotometry was also applied for the determination of some cephalosporins in binary mixtures [43]. A spectrophotometric method was reported for the determination of cefalexin bulk drug and its acid-induced degradation products [44]. UV spectrophotometry and difference UV spectrophotometry were applied to determine cefalexin in tablets. In addition, derivative spectrophotometry was used to determine the triethylammonium salt of cefotaxime in the presence of related compounds resulting from the synthesis.

Dissociation constants of cefepime and cefpirome were determined by UV spectrometry. Cefuroxime axetil and probenecid were simultaneously determined in solid dosage forms by UV spectrophotometric method. Derivative spectrophotometry was reported for the determination of cefprozil in pharmaceutical dosage forms in the presence of its alkali induced degradation products. Binary mixtures of cefalotin and cefoxitin were determined by first-derivative spectrophotometry. A spectrophotometric method has been developed for determination of cefadroxil in bulk powder and its pharmaceutical dosage forms based on the reaction of primary amine group with acetylacetone-formaldehyde reagent, which gives a yellow coloured chromogen. Another method was reported for the determination of cefadroxil in pharmaceutical dosage forms through mixing with sulfanilic acid; the absorbance was measured at 440 nm.

Thin-layer Chromatographic Methods: Qureshi *et al*., has determined Cefradine and cefalotin by spectrodensitometric method after contact with iodine vapors. Cephalosporins were applied to TLC plates coated with a mixture (2:1) of layered double hydroxide of aluminum(III) and magnesium(II) and silica gel G and developed with a range of mobile phases, the spots were detected with iodine vapours and the cephalosporin content was determined. Degradation products of ceftazidime, cefuroxime sodium and cefotaxime sodium were prepared by acid hydrolysis, mixtures of these drugs and their degradation products were analyzed by quantitative densitometric TLC. Some cephalosporins in phosphate buffer of pH 3.6 were spotted on TLC plates coated with silica gel with a fluorescent indicator or silica gel RP 18; the spots were visualized at 254 nm or with a color-forming agent.

Ceftriaxone, cefixme, cefotaxime, cefaclor and cefalexin were determined in their pharmaceutical dosage forms using HPTLC and the measurement of each spot was carried out at specified wavelengths using a scanner in absorbance/reflectance mode [45,46] reported a method for simultaneous determination of cefadroxil and cefalexin in pharmaceutical preparations using quantitative TLC. Cefalexin was analysed by HPTLC on silica gel F254 plates. The plates were scanned in reflectance mode at 263 nm [47]. Few methods have been reported for the quantification of Cephalexin single drug and in Combination by HPTLC [46-48].

The use of RP-HPLC procedures for determination in plasma, serum and urine has been reported [49-51]. Few HPLC methods also have been reported for quantification of Cephalexin [44, 52]. Some spectrophotometric and colorimetric methods also have been reported [53, 54].

However till now, no stability indicating method for estimation of Cephalexin has been reported Cefuroxime axetil and cefuroxime were determined using TLC densitometry after separation on silica gel using chloroform/ ethyl acetate/glacial acetic acid/water (4:4:4:1) as a mobile phase.

Dhanesar proposed a densitometric method for quantitation of some cephalosporins on a hydrocarbon impregnated silica gel HPTLC plate. Simultaneous determination of cefalexin and probencid in pharmaceutical preparation was performed by HPTLC using silica Gel 60 F254 HPTLC plate and they were detected at 254 nm. Also HPTLC method was used for the determination of ceftriaxone in injection solutions using butanol/acetonitrile/water (3:1:1) as developing solvent and detection at 254 nm [11].

Analysis of Cephalosporin in Biological Fluids:

High- Performance Liquid Chromatographic Methods: This technique is the most frequently applied technique for the determination of cephalosporins in biological fluids (blood, plasma, urine, cerebrospinal fluid, etc.), animal tissues, food, etc. summarises the recent HPLC reported methods for the analysis of cephalosporins in biological fluids, animal tissues, food, etc.

Capillary Electrophoretic Methods: Nine cephalosporins were determined using capillary zone electrophoresis after hydrodynamic injection on a fused-silica capillary and detection was performed at 210 nm Mrestani *et al*., proposed a CZE (Capillary Zone Electrophoresis) method for the determination of four cephalosporins after injection on a fused-silica capillary with detection at 270 nm and 30 kV separations potential. Cefixime and five of its metabolites were determined in human digestive tissues by high performance capillary electrophoresis on a fused-silica capillary tube with detection at 280 nm. CZE was used to determine β-lactam antibiotics, aminoglycosides, quinolones and tetracyclines in biological samples.

Cefotaxime and its deacetyl metabolite were determined by CZE using a fused-silica capillary with borate buffer pH 9.2 as electrolyte .CZE was used for the determination of four cephalosporins in clinical samples.

Micellar electrokinetic capillary chromatography (MEKC) Cefuroxime was determined in human serum by MEKC using a fused-silica capillary with 150mM sodium dodecyl sulfate in 20mM sodium phosphate and borate (pH 9.0) as electrolyte, with applied potential of 15 kV and detection at 274 nm.

Yeh *et al*., proposed a MEKC method for determination of ceftazidime in plasma and cerebrospinal fluid using Tris buffer with sodium dodecyl sulfate as background electrolyte and detection was performed at 254 nm. MEKC was used for determination of cefotaxime and it's deacetyl metabolite using a fused-silica capillary with phosphate buffer pH 8.0 containing 165mM sodium dedecylsulphate as separation electrolyte. Cefpirome was estimated in human microdialysis and plasma samples by MEKC.

The effect of various parameters such as pH, pre-concentration time, deposition potential, supporting electrolytes, possible interferences and other variables were investigated for the examined drugs. Statistical analysis was carried out for the obtained results. Good linearity's were obtained. The proposed method was successfully applied to the analysis of the studied drugs in their available pharmaceutical formulations [55] and in biological samples (serum and urine). The interference of some amino acids urea, ascorbic acid and some metal ions was investigated.

The developed Liquid chromatographic method with UV-Visible detection offers, sensitivity, precision and accuracy. It produces symmetric peak shape, good resolution and reasonable retention time for Ceftazidime and sulbactam sodium.

Moreover there is no pretreatment of the sample which makes the method simple and easy to perform. It can be used for the simultaneous determination of Ceftazidime and sulbactam sodium in the pharmaceutical companies and research laboratories for routine analysis and in plasma samples [56].

TABLE 1: DIFFERENT HPLC METHODS FOR THE ANALYSIS OF CEPHALOSPORIN WITH ANALYTICAL PARAMETERS

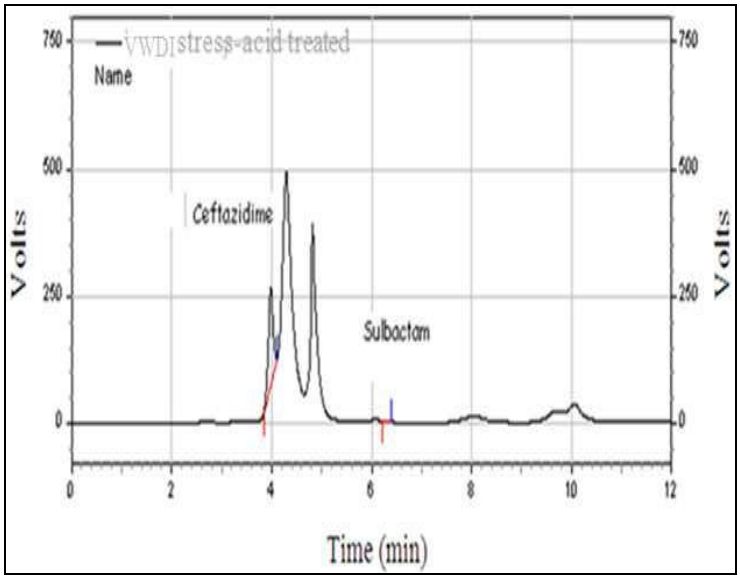
Method	Type of cephalosporin	Linearity	Precision % RSD	Accuracy & its range	LOD	LOQ	Retention Time	Reference
HPLC method	cefepime, cefixime and cefoperazone with seven cephalosporins	0.5, 1, 5, 10, 20, 30, and 50 ugmL−1	3.6–7.8 4.2–5.9 1.6–4.8 (inter day) 5.2–8.0 6.8–9.8 0.8–7.8(intra day)	0.50, 5.00 and 3 0.00ugmL^{-1}.	25, 10 and 15 ngmL^{-1}	80, 35 and 50 ngmL^{-1}	-	Emirhan Nemutlu *et. al*. [57]
HPLC method	cefetamet pivoxil in drug substance and powder forms	30.0–80.0 ug ml−1	1.71 – 1.51 % RSD	100.09%	1.03 ug ml^{-1} degradation	3.15 ug ml^{-1} Degradation	6.2 min	Lisoni M. Morsch *et.al*. [58]
HPLC method	Oral cephalosphorins Plasma(S-1090)	0.09–9 ug/ml	< 6 % RSD	0.09–0.9 ug/ml Recovery, 102.3%	-	0.09 ug/ ml	22 min	H. Fujitomo *et.al.* [59]
HPLC method	Oral cephalosphorins Urine(S-1090)	0.5–100 ug/ml	< 6 % RSD	0.9–9 ug/ml 95.8–100.3%	-	0.5 ug/ ml	23 min	H. Fujitomo *et.al.* [59]
HPLC method	cephradine in human plasma	0.2- 30 ug/ml	0.2 mg/ml was 4.9% (intra-assay)	0.2 - 30 ug/ml	-	0.2 and 30 ug/ ml	10 min	Virginia M. Johnson *et.al*. [60]
HPLC method stability indicating method	Cefazolin	1 to 50 µg/ml	% RSD 0.8033 and 0.5856 %	95 – 100 %.	0.1 ug/ml,	0.3 ug/ml	UV 254 nm	N Lalita et al.[76]
HPLC method	Ceftazidime and Sulbactam in Spiked Plasma	125-750 ppm for ceftazidime and 62.5-375 ppm for sulbactam sodium.	0.11 0.74	98.69±0.12 98.75±0.31	0.11 ppm	0.34 ppm	-	Masoom Raza Siddiqui *et.al*.[63]
HPLC method	Cephalosporin	5-50 – 500 ug/ml	0.993 – 100	93 to 101%.	0.2 to 1.0 ug/ml	-	4 to 6 min	Steven A.Singns et.al.[67]
HPLC method	Oral cephalosphorins Urine (S-1090)	0.5–100 ug/ml	Less than 6%	100.2–101.5%	-	-	-	H. Fujitomo *et al*.[59]
HPLC method	cefotaxime, ceftazidime and ceftriaxone Along with alkali induced degradation product and commercial injection.	5-20 ug/ ml.	RSD % of 0.91,2.66 and 1.83 for cefotaxime, ceftazidime and ceftriaxone.	98.6 , 100.1 , 97.6 % in degradation product and 104.9, 102.2, 100.3 In injection. Cefotaxime Ceftazidime Ceftriaxone respectively	0.25 u,g/ ml	-	270 nm using a diode array detector.	Mohammed E. Abdel-Hamid[68]
HPLC method Stability study	CEFPROZIL IN ORAL SUSPENSION CEFZIL	6.51 □ u/mL to 97.66 □ u/mL	0.40n1.60%		5.96 mg/mL	18.07 mg/mL	UV 280 nm	Anna Jeli—SKA *et al*.[69]
HPLC simultaneous estimation	ceftriaxone sodium and sulbactam sodium in injection dosage form	140-250µg/mL	97.65%	100.21± .50	3µg/mL	12 µg/mL	UV 230 nm	B. Palanikumar *et al*. [70]
HPLC simultaneous estimation	Cefixime and Cloxacillin in Tablets	160 -240 ug/mL for cefixime and 400 – 600 ug/mL cloxacillin	0.69 for cefixime and 0.77 for cloxacillin	99.99% and 102.24%.	-	-	UV 225 nm	G.Rathinavel *et al*.[71]
HPLC simultaneous method	Cefuroxime axetil	5-50 µg/ml	0.328 interday 0.545 intraday	100.976 ± 0.439	2.409 ug/ml	7.951 ug/ml	UV 225 nm	Mahima R. Sengar [72]
HPLC simultaneous method	Five cephalo Cefixime, cefaclore, cefadroxile, cephalexine, cephradine	Cefixime 10 - 100 mg/l Rest – 0.1 -	5.8 – 6.9 intraday 8.3 – 7.7 interday	Recovery 80 – 90 %	-	-	UV 240 nm	Joy A. Mcateer *et al*. [73]

HPLC simultaneous method	Cefixine and Dicloxacillin	Correlation coefficient 0.9959	% RSD 0.379	99 – 100 %	-	-	UV 220 nm	K.Kathiresan *et al.* [74]
HPLC method	Cefadroxil	Correlation coefficient 0.9941	% RSD 0.02	high	0.06 ppm	0.2 ppm	UV – vis 210 nm	Ravi Shankar Shukla *et al*. [75]
HPTLC method	Cefprozil in Tablet Dosage Form	400, -2000 ng/spot	RSD 1.48	101.04±1.78	133, ng/spot	400, ng/spot	Rf – 0.37-0.40	V. JAGAPATHI RAJU *et.al*.[61]
HPTLC determination of	ceftriaxone, cefixime and cefotaxime in dosage forms	125–500 ng	RSD: 1.12–2.91%	99.8 to 101.4%	Satisfactory	satisfactory	-	S. Eric-Jovanovic *et.al*.[64]
Stability Indicating HPTLC Method	Cephalexin in Bulk and Pharmaceutical	of 500–1500 ng	0.7267 % intra day and 1.3623 % Inter day	98.71%.	51.03 ng	154.64 ng	Rf, retardation factor, value–0.56	R. M. Jeswani *et.al*.[65]
UV spectrophotometric method	Cefuroxime in formulation	4-28 ug/ml	% RSD 0.36	99.97 ± 0.3969	1.3669 ug/ml	4.1421 ug/ml	281 nm	Santosh Shelke *et.al*.[62]
UV Spectrophotometric Method of	Cefuroxime Axetil in Bulk and Pharmaceutical Formulation	4-28µg/ml	99.50% (Intra-day precision) and 99.60%(Inter-day precision)	99.97 ± 0.3969	1.3669(g/ml)	4.1421 (g/ml)	281 nm	Santosh Shelke *et.al*.[62]
Electrophoresis method	EIGHT CEPHALOSPORIN	3–1000 µg mL^{-1}	0.6–1.6% intra day 0.5 - 1.8%, interday	100%	0.5–5 µg mL^{-1}			A. R. Solangi *et.al.* [66]

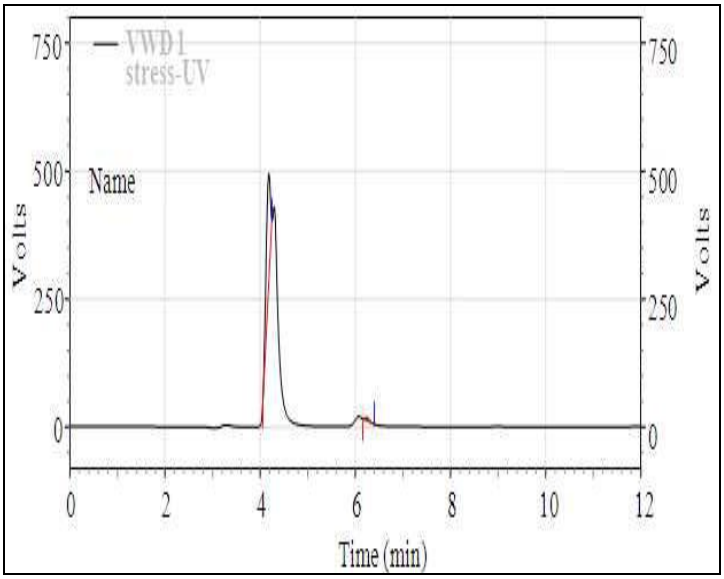
TABLE 2: DIFFERENT CHROMATOGRAPHIC CONDITIONS

Cephalosporins	Biological fluid/ Formulation	Method used	Column	Mobile phase	Detector	Reference
cefepime, cefixime and cefoperazone etc Seven cephalosphorins	Plasma & Amniatic food	HPLC method	XTerra C18 (250mm×4.6mm, 5_m i.d.) column	40mM phosphate buffer, pH 3.2, 18% MeOH, 0.85mLmin−1	photodiode array detector	Emirhan Nemutlu *et. al*. [57]
cefetamet pivoxil	Oral Formulation	HPLC method	C18 absorbosphere column (150×4.6 mm i.d., 5 _m particle size),	water–acetonitrile–methanol–phosphate buffer, pH 3.5 (50:35:10:5, v/v), flow rate of 1.5 ml min−1	UV detection at 254 nm.	Lisoni M. Morsch *et.al*. [58]
Oral cephalosporins Plasma(S-1090)	Human plasma	HPLC-column switching method	internal-surface reversed-phase pre-column and a C analytical column	pre-column was of 0.05 *M* phosphate buffer (KH PO –H PO , pH 2.5) analytical column was of 0.05 *M* phosphate buffer (pH 2.5)–methanol (80:20) mixture cont m*M* sodium 1-heptanesulfonate	UV detector	H. Fujitomo *et.al*.[59]
Oral cephalosporins Plasma(S-1090)	Urine	HPLC-column switching method	Two pre-columns packed with cyano and phenyl materials and a C analytical column were used for the urine assay	0.05 *M* phosphate buffer (pH 2.5)– methanol (75:25) analytical column was a 0.05 *M* phosphate buffer (pH 2.5)–methanol (80:20) mixture cont m*M* sodium 1-heptanesulfonate		H. Fujitomo *et.al.* [59]

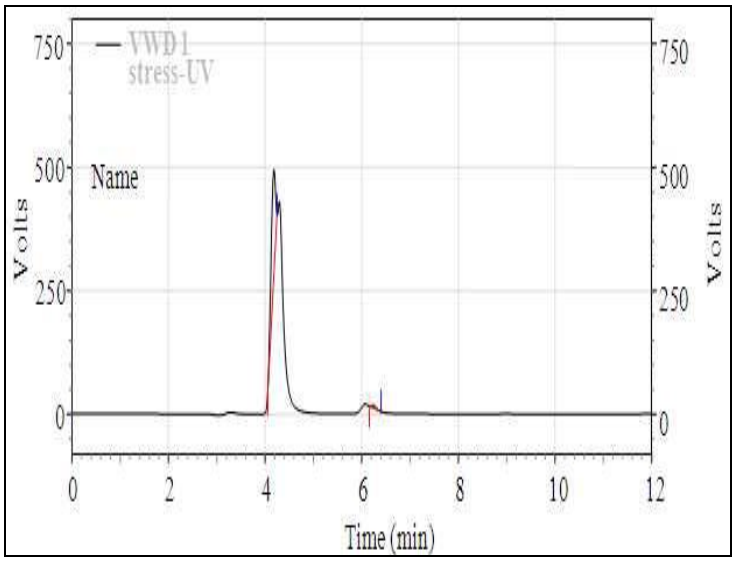
Ceftazidime and Sulbactam	Spiked Plasma and Combined Dosage form-Zydotam	HPLC method	Hypersil ODS C-18 column	Acetonitrile and tetrabutyl ammonium hydroxide adjusted to pH 5.0 with orthophosphoric acid in ratio 25:75.	UV detection at 230 nm	Masoom Raza Siddiqui *et al*.[56]
Ten Cephalosporins	Plasma	HPLC method	C-18 reverse-phase column,	0.01 M sodium acetate and Acetonitrile-methanol.	254-nm UV wavelength	STEVEN A. SIGNS *et al*.[67]
cephalosporins	pork and beef muscle tissues	HPLC method	SPE cartridges Bond Elut C18 6cc-500mg	pH 7 0.02M acetate buffer / methanol) in a gradient mode	252 nm UV wavelength	E. Verdon and P. Couëdor[
cephradine	Human Plasma	HPLC method	polymeric reversed-phase PLRP-S column	10.5% (v/v) acetonitrile in 20 m*M* ammonium dihydrogen orthophosphate (pH 2.75)	ultraviolet detection at 260 nm	Virginia M. Johnson *et al*.[60]
oral cephalosporin, cefmatilen hydrochloride hydrate, and its seven metabolites	Human and animal plasma and urine	HPLC method combination of ion-exch pre- column and (ODS) columns	Ion exchange precolumn and ODS column	Phosphate buffer, Methanol, acetonitrile and water.	UV detector 260 nm	I. Nishino *et al*.[78]
cefotaxime, ceftazidime and ceftriaxone	In presence of their alkali induced degradation products and in commercial injections	HPLC method	(150 mm X 6 mm ID) Schimpack GLC-ODS. 5 pm column	Mobile phase composed of acetonitrile-ammonium acetate buffer solution (0.1 M) in a ratio lo:90 (pH 7.5) with peak.	detection at 270 nm using a diode array detector	Mohammed E. Abdel-Hamid [68]
Cefprozil	Tablet Dosage Form	HPTLC method	silica gel G 60F254(20 cm x10 cm)	chloroform: methanol: toluene: diethyl amine: water in the ratio 4: 4.4: 3.2: 3: 0.8 v/v as mobile	Densitometer in absorbance mode at 286 nm.	V. Jagapathi raju *et.al* [61]



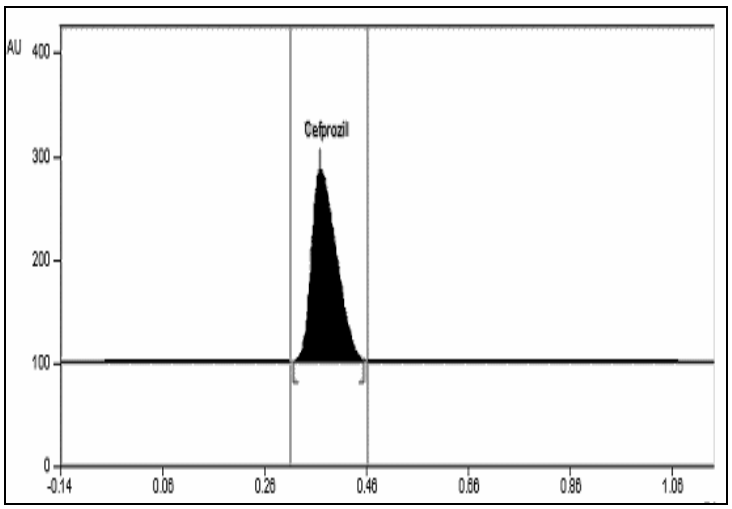
SPECTRA1: A TYPICAL LC CHROMATOGRAM OF A MIXTURE OF CEFTAZIDIME PENTAHYDRATE 500 ppm (PEAK NO. 1) AND SULBACTAM SODIUM, 250 ppm (PEAK NO. 2) ACHIEVED USING THE PROPOSED METHOD [56]



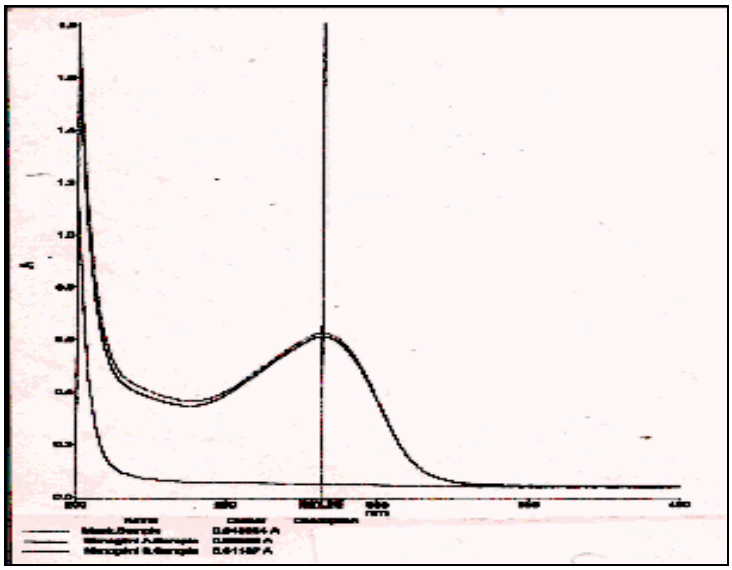
SPECTRA 2:- CHROMATOGRAM OF CEFTAZIDIME PENTAHYDRATE AND SULBACTAM SODIUM AFTER SUBJECTED TO ACID DEGRADATION [56]



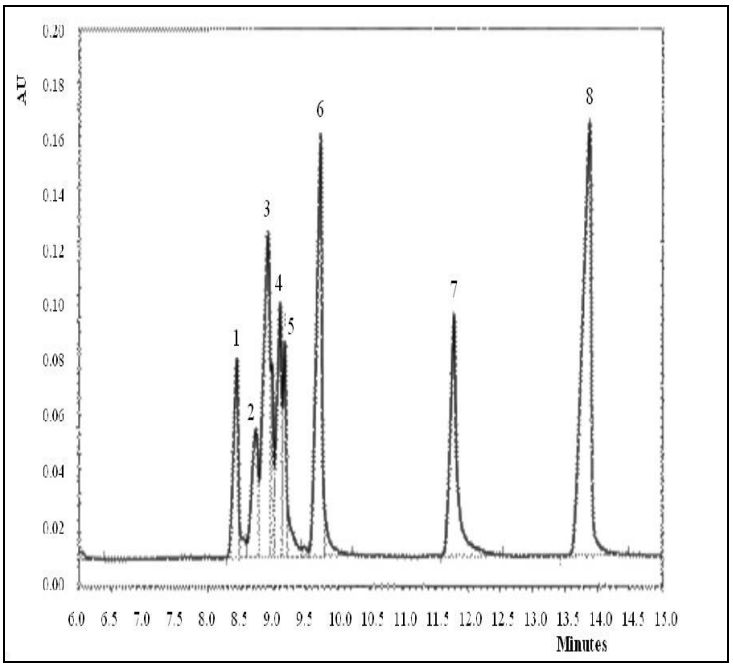
SPECTRA 3: CHROMATOGRAM OF CEFTAZIDIME PENTAHYDRATE AND SULBACTAM SODIUM AFTER SUBJECTED TO THERMAL DEGRADATION [56]



SPECTRA 4: A TYPICAL HPTLC DENSITOGRAM OF CEFPROZIL STANDARD [61].

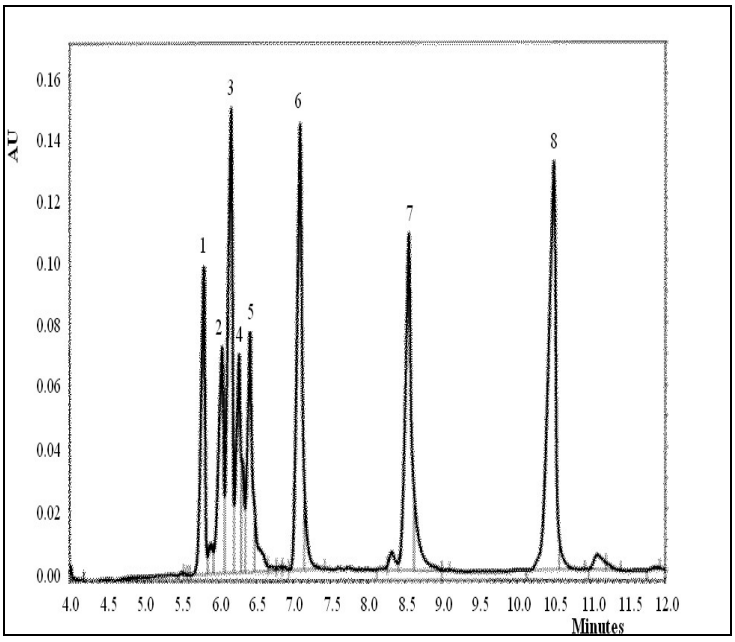


SPECTRA 5: DETERMINATION OF MAX OF CEFUROXIME AXETIL BY UV SCANNING



SPECTRA 6:- TYPICAL ELECTROPHEROGRAM OBTAINED FROM A MIXTURE OF THE CEPHALOSPORINS. CE CONDITIONS: APPLIED POTENTIAL 20 KV; BUFFER 50 MM SODIUM TETRABORATE, pH 9; DETECTION WAVELENGTH 214 NM.

1, CEFRADINE; 2, CEFTIZOXIME; 3, CEFOTOXIME; 4, CEFUROXIME; 5, CEFACLOR; 6, CEFADROXIL; 7, CEFTRIAXONE, AND 8, CEFIXIME [66]



SPECTRA 7:- TYPICAL ELECTROPHEROGRAM OBTAINED FROM A MIXTURE OF THE CEPHALOSPORINS. CE CONDITIONS: APPLIED POTENTIAL 30 KV; BUFFER 50 MM SODIUM TETRABORATE, pH 9; DETECTION WAVELENGTH 214 NM.

1, CEFRADINE; 2, CEFTIZOXIME; 3, CEFOTOXIME; 4, CEFUROXIME; 5, CEFACLOR; 6, CEFADRO-XIL; 7, CEFTRIAXONE AND 8, CEFIXIME [66].

DISCUSSION & CONCLUSION: This review contains different analysis methods includes some chromatographic methods especially HPLC, HPTLC and some capillary electrophoresis methods of analysis.
The different methods of analysis are specifically studied about the analytical parameters which include linearity, range, accuracy, precision, LOD, LOQ and robustness of the method along with combination of more than one antibiotic from formulation and biological fluids.

Analysis of antibiotics present in formulated and unformulated samples demands for a highly specific and rapid method as many antibiotics (e.g. β-lactams) have serious stability problems. This has made HPLC preferred over other traditional microbiological methods. Also chromatographic techniques are usually sensitive enough for most antibiotics as they achieve a limit of quantification (LOQ) of 0.3-0.5ug/ml. Sensitivity can be further enhanced by coupling it with fluorimetric, electrochemical or mass-spectrometric detection methods. By using different types of columns and varying combinations of solvent systems, scope of HPLC method can be further expanded to a wide range of samples, although reversed phase columns were used more frequently.

The purpose of this study is to find out the methods which are giving good results like precision. Some of these methods are used at very low concentration range of linearity. HPLC, HPTLC, UV spectrophotometric, capillary zone electrophoresis methods are commonly used for determining drugs in pre and final pharmaceutical products. Some reported methods are suitable for the identification and quantification of the cephalosporins at many ranges of analysis.

In conclusion, the different HPLC method has been successfully used on a routine basis and allows the quantification of the drug in pharmaceutical formulations and body fluids in a short analytical time.

These methods are sensitive, simple, fast, ease extraction procedure and possess excellent linearity and precision characteristics. These observations made it possible to anticipate the use of this method as an official procedure.

The data of validation also shows that the proposed methods are selective, linearity, precise and accurate with low LOD, LOQ values and adequate to determine the content of drug substance in formulated, non formulated products and in body fluids.

The HPTLC method by V. Jagapathi Raju *et al*., has shows a method for analysis of cephalosporin in tablets with nano gram level and high precision value. H. Fujitomo *et al*., proposed two methods for the determination of cephalosporins in oral suspension with little change in analysis range and variation in method.

The HPLC is more sensitive, linear, precision and high accurate method. Lisoni *et al*., showed that the analysis of cefatanet proxitil in drug substance and powder form with very low precision and high % of recovery. The LOD and LOQ value of the said method is also low concentration.

Masoom Raza *et al*., and Ravi Shankar *et al*., develop a method which can work on ppm level of concentration with high accuracy and precision with low LOD, LOQ value. Some HPLC methods can be used for the multi-component analysis with 7 - 10 cephalosporins at a time.

REFERENCES:

1. F. Kreuzig, in: J. Sherma, B. Fried (Eds.): Antibiotics in Hand book of TLC, Marcel Dekker, New York, 1996: 445.
2. J.D. Williams, K.G. Naber, A. Bryskier, N. Hoiby, I.M. Gould, P. Periti, H. Giamarellou, B. Rouveix: Int. J. Antimicrob. Agents 2001; 17: 443–450.
3. Bayer T, Zhou W, Holzhauer K, Schiigerl K.: Investigations of cephalosporin C production in an airlift tower loop reactor. Appl Microbiol Biotechnol, 1989; 30:26_/33.
4. Heim J, Shen YQ, Wolfe S, Demain AL.: Regulation of isopenicillin N synthetase by carbon source during the fermentation of cephalosporium acremonium. Appl Microbiol Biotechnol 1984; 19:232;/6.
5. Shalini Joshi: HPLC Separation of antibiotics present in formulated and unformulated samples, Journal of Pharmaceutical and Biomedical Analysis, 2002; 28: 795-809.
6. The European Pharmacopeia, fourth ed., Council of Europe, 2002.
7. The United States Pharmacopeia XXX and NF XXV, American Pharmaceutical, 2007.
8. Salwa R. El-Shaboury, Gamal A. Saleh , Fardous A. Mohamed, Azza H. Rageh: Analysis of cephalosporin antibiotics, Journal of Pharmaceutical and Biomedical Analysis 2007;45: 1–19.
9. S. Atinoz, D. Ozer, A. Ozer, A. Temized, N. Yuksel : Determination of ceftriaxone in aqueous humor and serum samples by differentialpulse adsorptive stripping voltammetry, *Analyst* ,1994; 119:1575- 1577.

10. Al-Momani IF: Spectrophotometric determination of selected cephalosporins in drug formulations using flow injection analysis. *J Pharm Biomed Anal*, 2001; 25, 751-757.
11. S.S. Zarapkar, S.A. Shinvalkar, A.A. Dhanvate, P.M. Deshpande, S.S. Kolte: High-performance thin-layer chromatographic determination of ceftriaxone sodium from pharmaceutical preparations, *Indian- Drugs* 1995; 32: 232-235.
12. M.C. Nahata, R.S. Morosco: Measurement of ceftazidime arginine in aqueous solutions by HPLC, *J. Liq. Chromatogr*. 1992;15: 1507- 1511.
13. J. Parasrampuria, V. Das-Gupta: Quantitation of cefadroxil in pharmaceutical dosage forms using high performance liquid chromatography, *Drug Dev. Ind. Pharm.*,1990; 16: 1435-1440.
14. C.E. Fasching, L.R. Peterson, D.N. Gerding: High pressure liquid chromatographic analysis for quantitation of BMY 28142 and ceftazidime in human and rabbit serum, *J. Liq. Chromatogr*. 1986;9: 1803-1814.
15. VS. Kartashov, AI. Chemyshev, R.D. Poliser, S.E. Esipov, A.P. Arzamastsev: NMR spectrometry in pharmaceutical analysis. V. Proton NMR spectroscopic identification of cephalosporin antibiotics, Khim-Farm.-Zh. 1990;24: 85-88.
16. 16] B. Ogorevc, V. Hudnik, S. Gomiscek: Polarographic analysis of some cephalosporin antibiotics, Fresenius Z. Anal. Chem. 1988;330: 59- 64.
17. M.A. Abounassif, N.A.A. Mian, M.S. Mian, Analytical profile of ceftazidime, in: K. Florey (Ed.), Analytical Profiles of Drug Substances, vol. 19, Academic Press, San Diego, CA, 1990: 95-121.
18. Nakagawa, T., J. Haginka, K. Yamaoka, and T. Uno: High speed liquid chromatographic determination of cephalexin in human plasma and urine. J. Antibiot.1978; 31:769-775.
19. Nilsson-Ehle, I.: High-pressure liquid chromatography as a tool for determination of antibiotics in biological fluids. Acta Pathol. Microbiol. Scand., 1977: 259:61-66.
20. Nilssou-Ehle, I., T. T. Yoshikawa, M. C. Schotz, and L. B. Guze: Quantitation of antibiotics of high-pressure liquid chromatography: cephalothin. Antimicrob. Agents Chemother.1978: 13:221-227.
21. Rotschafer, J. C., K. B. Crossley, T. S. Lesar, D. Zaske, and K. Miller: Cefaclor pharmacokinetic parameters: serum concentrations determined by a new high-performance liquid chromatographic technique. Antimicrob. Agents Chemother. 1982; 21:170-172.
22. Smith, R. V., and J. T. Stewart. : Textbook of biopharmaceutical analysis, 1981: 79-92.
23. Waterman, N., L. Scharfenberger, and M. J. Taff. : Rate of binding of antibiotics to canine serum protein. Antimicrob. Agents Chemother, 1974; 5:294-295.
24. Wold, J. S.: Rapid analysis of cefazolin in serum by high-pressure liquid chromatography. Antimicrob. Agents Chemother. 1977; 11:105-109.
25. Wold, J. S., and S. A. Turnipseed. : Determination of cephaloridine in serum and tissue by high-pressure liquid chromatography. J. Chromatogr. 1977; 136:170-173.
26. Jiang, E. and C. Hu,: Determination of ceftazidime and impurities using high performance liquid chromatography. Se Pu,2008; 26: 75-79. http://www.ncbi.nlm.nih.gov/pubmed /18438029
27. Guitton, J., A. Laffont, J. Bruzeau, L.R. Mingret, J. Bonnefoy and M. Bureau, : Determination of ceftazidime in plasma using high-performance liquid chromatography and electrochemical detection. Application for individualizing dosage regimens in elderly patients. J. Chromatogr. B. Biomed. Sci. Applied, 1998; 719: 151-157. http://www.ncbi.nlm.nih.gov/pubmed/9869375
28. Ayerton, J.: Assay of ceftazidime in biological fluids using high-pressure liquid chromatography. J. Antimicrob. Chemother., 1981; 8: 227-231. http://jac.oxfordjournals.org/cgi/content /abstract/8/
29. Barnes, A.R.: Determination of Ceftazidime and pyridine by HPLC: Application to a viscous eye drop formulation. J. Liquid Chromatogr.1995; 18: 3117-3128. http://www.informaworld. com/smpp /content~db=a
30. Ye, G., X. Cai, B. Wang, Z. Zhou, X. Yu, W. wang, J. Zhang, Y. Wang, J. Dong and Y. Jiang, : Simultaneous determination of vancomycin and ceftazidime in cerebrospinal fluid in craniotomy patients by high-performance liquid chromatography. J. Pham. Biomed. Anal., 2009; 48: 860-865.
31. Isla, A., A. Arzuaga, J. Maynar, A.R. Gascon, M.A. Solinis, E. Corral and J.L. Pedraz: Determination of ceftazidime and cefepime in plasma and dialysate-ultrafiltrate from patients undergoing continuous veno-venous hemodiafiltration by HPLC. J. Pharm. Biomed. Anal., 2009; 39: 996-1005. http://www.ncbi.nlm.nih.gov/pubmed/16026959
32. F.J. Muhatadi, M.A.A. Hassan, Cefotaxime, in: K. Florey (Ed.): Analytical Profiles of Drug Substances, vol. 11, Academic Press, San Diego, CA, 1982: 139-168.
33. J. Weismuller, A. Chanady,: Quantitative multicomponent analysis of complex mixtures by means offull spectrum quantitation and principal component analysis, Trends Anal, Chem.,1992; 11: 86-90.
34. M. Simonian: Quantitative analysis of complex mixtures with multicomponent analysis by full spectrum quantitation (FSQ). Part 2: Thequantitation of steroid hormone mixtures, Tech. Informatiom Bull., Beckman Instruments Inc., 1992.
35. M.E. Abdel-Hamid, SM. Al-Saidan,: Determination ofcephalexin and cephradine mixtures using full spectrum quantitation (FSQ) method for multicomponent analysis, Alex. J. Pharm. Sci. 10 1996: lOl- 105.
36. S.M. Al-Saidan, M.E. Abdel-Hamid, A.A. Abdel-Aziz, : Determination of chloramphenicol in pharmaceutical formulations using spectrophotometric full spectrum quantitation (FSQ) and high performance liquid chromatography (HPLC), Alex. J. Pharm. Sci. 10 1996: 173-178.
37. M.E. Abdel-Hamid, S.M. Al-Saidan,: Simultaneous multicomponent analysis of two- and three-component mixtures of drugs by spectrophotometric full spectrum quantitation (FSQ), Alex. J. Pharm. Sci. 11 ;1997: 111-117.
38. Rodenas, V., M.S. Garcia, C. Sanchez-Pedreno and M.I. Albero, : Flow-injection spectrophotometric determination of frusemide or sulphathiazole in pharmaceuticals. J. Pharm.Biomed. Anal., 1997; 15: 1687-1693.
39. K. Kelani, L. I. Bebaway and L. A. Fattah, J.:Stability-indicating spectrophotometric and densitometric methods for determination of some cephalosporins" AOAC. Int., 81, 1998; 386-396.
40. Moreno, A.D.H. and H.R.N. Salgado, : Spectrophotometric determination of ceftazidime in pharmaceutical preparations using neocuproin as a complexing agent. Anal. Lett.,(2008; 41: 2143-2152.http://www.informaworld.com/smpp/1356477
41. Marwa, S.E., S. Abdalla, M.N. Elbolkiny and M.H. Khalil, : - Spectrophotometric determination of aciclovir, ceftazidime pentahydrate, famotidine and isoxsuprine hydrochloride by ternary complex formation with eosin and Cu(II). Zhonghuá Yáoxué Zázhì, 2003; 55: 481-490.http://cat.inist.fr/?aModele =afficheN&cpsidt=16105994
42. Hiremath, B. and B.H. Mruthyunjayaswamy, : Development and validation of spectrophotometric methods for determination of

ceftazidime in pharmaceutical dosage forms. Acta Pharm. 2008; 58: 275-85. http://www.ncbi.nlm.nih.gov/pubmed /19103564
43. B. Morell: Simultaneous determination of ceftriaxone and streptomycin by ratio spectra 2nd derivative and zero-crossing 3rd derivative spectrophotometry, Talanta 41 1994; 673.
44. Campins Falco P.: Comparative study on the determination of cephalexin in its dosage forms by spectrophotometry and HPLC with UV-vis detection. Mikrochimica acta, 1997; 126, 207– 215.
45. S. Eric-Jovanovic, D. Agbaba, D. Zivanov-Stakic and S. Vladimirov; S.; HPTLC determination of ceftriaxone, cefixime and cefotaxime in dosage forms; online journal Biomed. Anal. 1999; 20: 557–564.
46. Agbaba D. et al.: HPTLC assay of cephalexin and cefaclor in pharmaceuticals. Biomedical Chromatography, 1998; 12: 133-135.
47. Coran S.A. *et. al.:* Development of a densitometric method for the determination of cephalexin as an alternative to the standard HPLC procedure., Journal of Pharmaceutical and Biomedical Analysis.1998; 18, 271–274.
48. Bhooshan R. and Prashad V.: Separation and Identification of Some Cephalosporin's on Impregnated TLC Plates., Biomedical Chromatography, 1996; 10, 258–260.
49. Najib N. M. *et. al*. : High performance liquid chromatographic analysis of cephalexin in serum and urine., Journal of Clinial pharmacy and therapeutics, 2008; 12, 419-426.
50. Zendelovska D. *et. al*.: Simultaneous quantification of cefaclor and cephalexine in blood plasma using high-performance liquid chromatography with UV detection., Acta Pharm, 2002; 52, 243–250.
51. 51) Lee Y.J. and Lee H.S.: Simultaneous determination of cefoxitin, cefuroxime, cephalexin and cephaloridine in plasma using HPLC and a column-switching technique. Chromatographia, 1990; 30, 80–84.
52. 52) Argekar A. P. et. al.: Simultaneous determination of Cephalexin and Carbocisteine from capsules by reverse phase high performance liquid chromatography (RP - HPLC)., Analytical Letters, 1997; 30, 821-831.
53. Prayanka P. and Suresh P.: Development of colorimetric method for cephalexin in dosage forms. Asian Journal of Pharmaceutics, 2008; 2, 120–122.
54. Patel S.A. *et. al*.: Spectrophotometric methods for the estimation of Cephalexin in tablet dosage forms. Indian Journal of Pharmaceutical Sciences, 2006; 68, 278-280.
55. O. A. Farghaly, O. A. Hazzazi, E. M. Rabie and M. Khodari.: Determination of Some Cephalosporins by Adsorptive Stripping Voltammetry; Int. J. Electrochem. Sci., 2008; 3: 1055 – 1064.
56. Masoom Raza Siddiqui, Abu Tariq, Manu Chaudhary, K. Dinesh Reddy, Prithvi Singh Negi, Jitendra Yadav, Nitya Srivastava, Sanjay Mohan Shrivastava and Rajkumar Singh : Development and Validation of High Performance Liquid Chromatographic Method for the Simultaneous Determination of Ceftazidime and Sulbactam in Spiked Plasma and Combined Dosage form-Zydotam; American Journal of Applied Sciences , 2009; 6 (10): 1781-1787.
57. Emirhan Nemutlu, Sedef Kır, Doruk Katlan, M. Sinan Beksac, : Simultaneous multiresponse optimization of an HPLC method to separate seven cephalosporins in plasma and amniotic fluid: Application to validation and quantification of cefepime, cefixime and cefoperazone, Talanta ,2009; 80 117–126.
58. Lisoni M. Morsch, Celso F. Bittencourt , Marineˆs J. e Souza , Julie Milano : LC method for the analysis of cefetamet pivoxil hydrochloride in drug substance and powder for oral suspension, Journal of Pharmaceutical and Biomedical Analysis , 2002;30 , 643–649
59. H. Fujitomo, T. Nagaoka, I. Nishino, T. Umeda : Determination of a new oral cephalosporin, S-1090, in human plasma and urine by direct injection high performance liquid chromatography with ultraviolet detection and column switching; Journal of Chromatography B, 1999; 728 125–131.
60. Virginia M. Johnson, John P. Allanson, Roger C. Causon: Determination of the cephalosporin antibiotic cephradine in human plasma by high-performance liquid chromatography with ultraviolet detection; Journal of Chromatography B, 2000; 740 ,71–80.
61. V. Jagapathi Raju and J. V. L. N. Seshagiri Rao: High Performance Thin Layer Chromatographic Method for Estimation of Cefprozil in Tablet Dosage Form; E-Journal of Chemistry 2008; Vol. 5, No.3, pp. 427-430.
62. Santosh Shelke, Santosh Dongre, Amit Rathi, Dinesh Dhamecha, Saifee Maria and Mohd Hassan G Dehghan Development and Validation of UV Spectrophotometric Method of Cefuroxime Axetil in Bulk and Pharmaceutical Formulation. Asian J. Research Chem. 2009; 2(2):23
63. Masoom Raza Siddiqui*, et al*.: Development and Validation of High Performance Liquid Chromatographic Method for the Simultaneous Determination of Ceftazidime and Sulbactam in Spiked Plasma and Combined Dosage form-Zydotam; American Journal of Applied Sciences, 2009; 6 (10): 1781-1787.
64. S. Eric-Jovanovic, D. Agbaba, D. Zivanov-Stakic and S. Vladimirov; S. ; HPTLC determination of ceftriaxone, cefixime and cefotaxime in dosage forms; online journal
65. R. M. Jeswani, P.K. Sinha, K.S.Topagi , M. C. Damle; A Validated Stability Indicating HPTLC Method for Determination of Cephalexin in Bulk and Pharmaceutical Formulation; International Journal of PharmTech Research July-Sept 2009; Vol.1, No.3, pp 527-536.
66. A. R. Solangi, S. Q. Memon, M. Y. Khuhawar, and M. I. Bhanger: Quantitative Analysis Of Eight Cephalosporin Antibiotics In Pharmaceutical Products And Urine By Capillary Zone Electrophoresis; Acta Chromatographica, 2007: 19
67. Steven A. Signs, Thomas M. File, And James S. Tan: High-Pressure Liquid Chromatographic Method for Analysis of Cephalosporins; Antimicrobial Agents and Chemotherapy, Nov. 1984; 26(5): 652-655.
68. M.E. Abdel-Hamid, S.M. Al-Saidan: Simultaneous multicomponent analysis of two- and three-component mixtures of drugs by spectrophotometric full spectrum quantitation (FSQ), Alex. J. Pharm. Sci.,1997;11: 111-117.
69. Anna Jeli—Ska, Beata Medenecka, Marianna Zaj. C And Malgorzata Knajsiak: The Stability Of Cefprozil In Oral Suspension Cefzil, Acta Poloniae Pharmaceutica n Drug Research,2008; Vol. 65 No. 2 : 261- 265.
70. B.Palanikumar, A.Thenmozhi, And D.Sridharan: An RP-HPLC Method For Simultaneous Estimation Of Ceftriaxone Sodium And Sulbactam Sodium In Injection Dosage Form; International Journal of Pharmacy and Pharmaceutical Sciences, 2010; Vol.2 Issue 3, 34 -38.
71. G. Rathinavel, P.B. Mukherjee, J. Valarmathy: A Validated RP – HPLC Method for Simultaneous Estimation of Cefixime and Cloxacillin in Tablets; CODEN ECJHAO E-Journal of Chemistry,(July 2008; Vol. 5, No.3, pp. 648-651.
72. Mahima R. Sengar, Santosh V. Gandhi , Upasana P. Patil, Vivek S. Rajmane, :Reverse Phase High Performance Liquid Chromatographic Method For Simultaneous Determination Of Cefuroxime Axetil And Potassium Clavulanate In Tablet Dosage Form; International Journal of Chem Tech Research CODEN(USA): IJCRGG,2009; Vol.1, No.4, pp 1105-1108.

73. Joy . McAteer, Mark F. Hiltke, B. Michael Silber, and Robert D. Faulkner: Liquid chromatographic Determination of Five Orally Active Cephalosphorine- Cefixime, Cefaclor, Cefadroxil, Cephalexin and Cephradine- in Human serum. Clin. Chem. 33/10, 1987: 1788-1790.
74. K.Kathiresan, R.Murugan, M. Shahul Hameed, K. Gokula inimai1 and Taranath kanyadhara: Analytical Method Development and Validation of Cefixime and Dicloxacillin Tablets by Rp-Hplc; Rasayan J Chem,2009; Vol 2, No. 3: 588-592.
75. Ravi Shankar Shukla, S Pandey, Rajesh Bargale, Asha Pateland, M Tiwari: Novel HPLC analysis of cefadroxil in bulk formulation. Asian Journal of Pharmaceutics, Nov. 2010, IP: 41.252.163.144.
76. N Lalitha, Pai PN Sanjay, MG Vyshak and Uvesh Kadri: Stability-Indicating Reverse Phase HPLC Method for the Determination of Cefazolin; Tropical Journal of Pharmaceutical Research, February 2010; 9 (1): 45-50.
77. Deana C. Hurum, Brian M. De Borba, Jeffrey S. Rohrer: Dionex Corporation, Sunnyvale, CA USA, Determination HPLC Determination of Impurities in the Cephalosporin Antibiotic Cefepime by Ion Chromatography and HPLC , Dionex Application note,
78. I. Nishino, H. Fujitomo, T. Umeda : Determination of a new oral cephalosporin, cefmatilen hydrochloride hydrate, and its seven metabolites in human and animal plasma and urine by coupled systems of ion-exchange and reversed-phase high-performance liquid chromatography, Journal of Chromatography B, 2000; 749 , 101–110.

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