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Review

HPLC separation of antibiotics present in formulated and unformulated samples

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Abstract

Analysis of antibiotics in formulated and unformulated samples demand a highly specific and rapid method as many antibiotics (e.g. β-lactams) have serious stability problems. HPLC techniques can provide a valuable tool for generating highly pure preparations for characterizing the antimicrobial activities. In the present review article, column and mobile phase conditions for the various classes of antibiotics viz. penicillins, cephalosporins, macrolides, tetracyclines, aminoglycosides, quinolones, rifamycins etc. have been presented from April 1998 to November 2000. A brief discussion on chemical structure, spectrum of activity and action mechanism of each class has also been given.

Keywords: Reviews; HPLC; Antibiotics; Pharmaceutical analysis; Penicillins; Cephalosporins; Macrolides; Tetracyclines; Aminoglycosides; Quinolones

1. Introduction

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 40 000 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 means 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].

Structures of antibiotics vary widely, therefore they have been classified in many groups. However, members of each group resemble each other. Their origin and instability leads to a situation in which small amounts of structurally related compounds and byproducts may be present together. It is very difficult to determine small amount of degradation product in a vast excess of parent drug. It is also difficult to analyze interference from excipients during the assay of raw materials

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and formulated products. These problems act as a hindrance in the analysis of antibiotics; which is required in many fields like optimization of fermentation, product isolation, process control and determining their optimal dosages and also in determining their concentration in body fluids. Commonly used methods of quantitative analysis of antimicrobial compounds are chromatographic methods, microbiological assays and radioimmuno assays. Microbiological methods can assay the parent drug together with its active metabolite(s), which is what is required by the bioavailability definition set out in the European Union (EU) and U.S. Food and Drug Administration (FDA) guidelines on bioavailability and bioequivalence [2,3]. However, these methods [4,5] employ microorganisms that are very sensitive to the given drug, they often lack specificity as compared to chromatographic methods specially Gas chromatographic (GC) and HPLC. Other immunoassays recently reviewed by Hage [6] for their application in clinical and biomedical sci-

Table 1
List of review articles published recently on antibiotics LC separation

Name of the review article	No. of references cited	Ref.
Chromatography as an analytical tool for selected antibiotic classes: a reappraisal addressed to pharmacokinetic applications	55	[12]
Analysis of antibiotics by liquid chromatography–mass spectrometry	88	[13]
High performance liquid chromatographic analysis of aminoglycoside antibiotics	42	[14]
Analysis of macrolide antibiotics	140	[15]
Aminoglycoside antibiotics—two decades of their HPLC bioanalysis	49	[16]
New advances in analytical methods on fluoroquinolone antibacterial agents	50	[17]

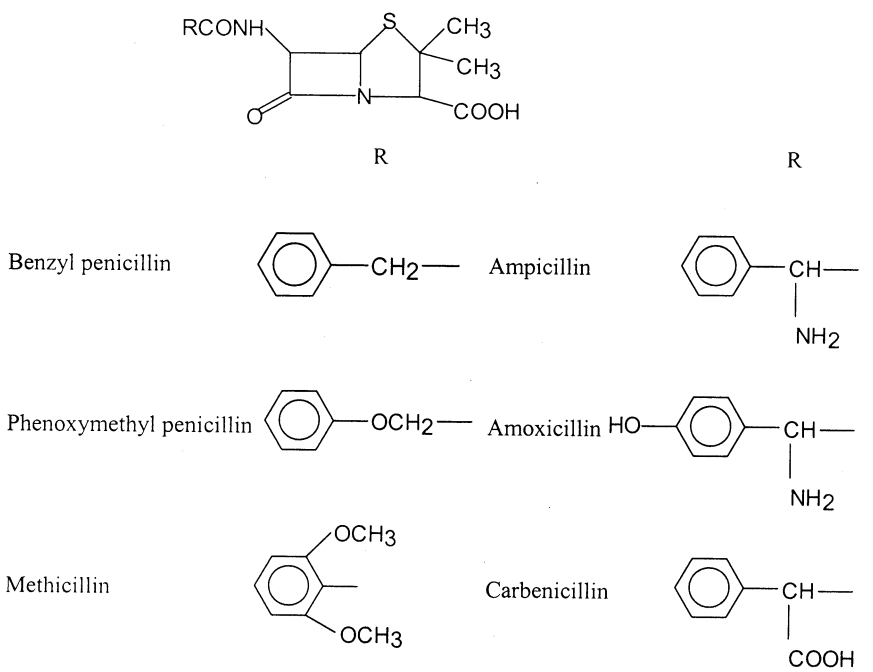


Fig. 1. Chemical structure of some important penicillins.

Table 2
HPLC separation of penicillin and related antibiotics

Name of compound	Stationary phase	Mobile phase	Ref.
Benzyl penicillin	C_{18}	0.5 M-Phosphate buffer at pH 3.5/MeOH/H_2O (1:3:6, solvent A; 1:5:4, solvent B; A:B, 7:3) as mobile phase	[21]
Amoxicillin sodium and its related compounds	5 mm Supelcosil ABZ+plus column	0.1%TFA of pH 2.1/MeCN (93:7, solvent A; 4:1, solvent B); gradient elution from 100% A to 40% A in 2 min	[22]
Isoxazolyl penicillins (analysis of degradation products obtained after hydrolysis)	Phenomenex resolve C_{18}	Various ratios of 18–21% MeCN/20 mM-KH_2PO_4 buffer of pH5 containing 10 mM-tetramethyl ammonium chloride	[23]
Assay of procaine and benzylpenicillin in procaine benzylpenicillin	ODS	Mobile phase consisting of KH_2PO_4 tetrabutylammonium hydroxide solution (pH 7.2) and MeCN	[24]
Benzathine benzyl penicillin	Hypersil BDS	Phosphate buffer (pH 3.5)/MeOH/H_2O (2:7:11)	[25]
Mezlocillin sodium and sulbactam sodium	μ-Bondapak C_{18}	5 mM-Tetrabutyl ammonium hydroxide (adjusted to pH 5 with 0.1 M-H_3PO_4)/MeCN (17:8)	[26]
Amoxicillin	Lichrosorb RP-C_{18}	50 mM-KH_2PO_4 containing 45% KOH buffer of pH 5/MeCN (24:1)	[27]
Tazobactam and Tazocin	Spherisorb C_{18}	H_2O/MeOH/2.5 mM-Tetrabutyl ammonium hydroxide (107:90:3) with pH adjusted to 3.5 with 50% H_3PO_4	[28]
Amoxycillin sodium and potassium clavulanate	Platinum EPS C_{18}	0.05% TFA of pH 2.6 and 0.05% TFA/MeCN (4:1)	[29]
Ampicillin and probenecid	C_{18} Lichrosorb	1% Ortho phosphoric acid (760 ml) and MeOH (240 ml)	[30]
Amoxicillin and ambroxol hydrochloride	μ Porasil	0.01 M-KH_2PO_4/MeCN (3:2) adjusted to pH 3 with 5% ortho phosphoric acid	[31]
Piperacillin sodium and tazobactam sodium	Auto science Kromasil C_{18}	MeOH/H_2O/0.2 M-sodium dihydrogen phosphate/10% tetrabutyl ammonium hydroxide (510:432:50:8) adjusted to pH 5.5 with 10% H_3PO_4	[32]

ences also suffer from disadvantage of non-versatility to be applicable in routine chemical analysis. GC methods [7,8] are fast and specific, but they require elevated operating temperatures which may occasionally cause thermal degradation of underivatized drug, and therefore frequent necessity for derivatization to increase volatility and to improve chromatographic behavior. These methods are generally inapplicable for the analysis of highly polar substances (e.g. sulphate and glucuronide conjugates of antibiotics and their metabolites or of substances of high molecular weight [9]. TLC [10] and its modern modification HPTLC [11] are used in various fields of antibiotic chemistry but, with their high detection limit, they were not preferred over HPLC.

High resolving power of HPLC serves as a particularly important method for isolation and purification of antibiotics. In addition, since the purified molecule is routinely used in antimicro-

bial assays and it is critical that antibiotic preparations being tested be devoid of artifactual (antimicrobial) components introduced during purification. In this regard, HPLC techniques can provide a valuable tool for generating highly pure preparations for characterizing the antimicrobial activities. Also HPLC with its ability to analyze both volatile and non-volatile compounds, to determine ultratrace to preparative to process scale separations, may be employed in clinical laboratories.

Several assays based on HPLC have been reported in the last few years/decades. Since many antibiotics contain ionizable groups, the earliest separations were obtained by ion-exchange chromatographic methods but nowadays reversed phase methods on bonded phase material have been used. Many review articles published recently (Table 1) have covered literature on HPLC analysis of antibiotics. Synthetic compounds having antimicrobial action (e.g. quinolone and sulphonamides) have also been discussed [12–17].

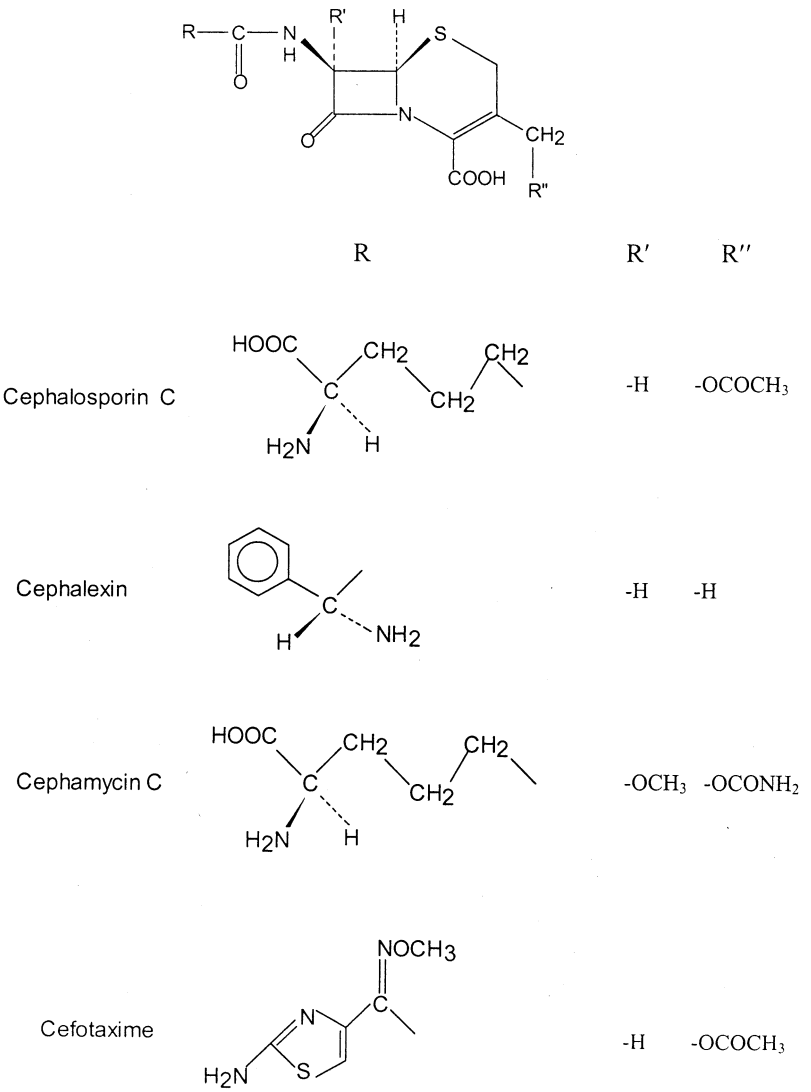
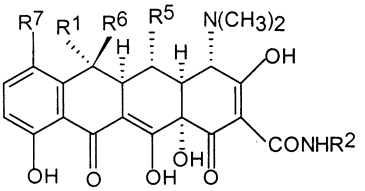


Fig. 2. Chemical structure of cephalosporins.

Table 3
HPLC separation of cephalosporins

Name of compound	Stationary phase	Mobile phase	Ref.
Cefalexin	Spherisorb C_{18}	10 mM-KH_2PO_4 buffer/MeCN/MeOH (45:40:1) with pH adjusted to 4.5 with H_3PO_4	[34]
Cefotaxime, Ceftazidime and ceftriaxone	Shimpack GLC-ODS	MeCN/0.1 M-ammonium acetate (1:9) adjusted to pH 7.5 with ammonia solution	[35]
Cefazolin, cefadroxil, cephalexin, ampicillin and cephradine	Phenomenex C_{18}	–	[36]
D-phenylglycine-containing cefotaxime double esters (Isomer separation)	Lichrospher 100RP-18	Aq. 0.5%TFA/MeCN (3:1)	[37]
Carbanepem analysis of degradation products	Prodigy ODS2	5 mM-Hexadecyltrimethyl ammonium bromide in THF/MeCN/25 μM-ammonium phosphate buffer of pH 5 (1:4:5)	[38]
Cefotaxime and ceftriaxone	Lichrosorb RP-18	MeCN/67 mM-KH_2PO_4 buffer adjusted to pH 2.65 with H_3PO_4 (1:4)	[39]
Ceftriaxone	Kromasil C_{18}	MeCN/tetrabutyl ammonium bromide/phosphate buffer of pH 7/H_2O (400:4:55:795)	[40]
Cefaclor, cephalexin, isoniazid, minocycline and pyrazinamide	ODS silica	25 mM-Tetrabutylammonium hydrogen sulphate/MeCN/MeOH (48:1:1)	[41]
Cephalexin, cefotaxime sodium and salbutamol sulfate	Supelosil ODS	MeOH/phosphate buffer of pH 7 (3:2)	[42]
Cefaclor	Irregular-HC_{18}	H_2O/MeOH/3.8 % sodium acetate (1380:591:29)	[43]
Cephalexin	μ Bondapak C_{18}	Phosphate buffer of pH 7.4/MeOH (3:1)	[44]
Ceftazidine	Alltima C_{18}	43.5 mM-ammonium dihydrogen phosphate buffer adjusted to pH 3.9 with H_3PO_4/MeCN (93:7)	[45]
Cefetamet pivoxyl hydrochloride	Nova-Pack C_{18}	H_2O/MeCN/MeOH/phosphate buffer [containing 5 mg/ml NaH_2PO_4 and 35 mg/ml KH_2PO_4] (100:72:19:9)	[46]
Mixture of six cephalosporins	Hypersil ODS C_{18}	50 mM-KH_2PO_4 buffer of pH 3.4/MeCN (875:125)	[47]
Cephalexin	YWG C_{18}	15 mM-Acetate buffer of pH 4/MeOH/MeCN (60:30:6)	[48]

Efforts have been made to further update the current literature from April 1998 to November 2000 in the present review. Attention has been paid on column and mobile phase conditions for the various classes of antibiotics. However, a brief introduction about each group of antibiotics has also been given. References dealing only with unformulated and formulated products have been discussed here, and those covering biological samples will be discussed in future section. Great care has been taken in compiling the literature however any omission in the references are purely inadvertent and are highly regretted.



	R^1	R^2	R^5	R^6	R^7
Chlortetracycline	H	H	OH	CH_3	Cl
Oxytetracycline	H	OH	OH	CH_3	H
Tetracycline	H	H	OH	CH_3	H

Fig. 3. Chemical structure of some important tetracyclines.

2. Penicillins

Penicillin was the first microbial metabolite to distinguish between toxicity to the bacterial cell and toxicity to the mammalian host to permit its use in the systemic treatment of infections caused by gram-positive and -negative organisms in humans and animals. The basic structure of penicillin nucleus includes a β-lactam ring fused through nitrogen and adjacent tetrahedral carbon to a second heterocycle, which in natural penicillin is a five-membered thiazolidine ring (Fig. 1). Semi-synthetic penicillins are produced starting from 6-aminopenicillanic acid, which are obtained from culture of *Penicillium chrysogenum*. These molecule are more resistant to β-lactamase e.g. ampicillin, oxacillin etc. [18].

Penicillin and other β-lactams (cephalosporins) inhibit the synthesis of essential structural components of bacterial cell wall i.e. peptidoglycan which are absent in mammalian cells [19,20]. Thus host cell metabolism remains unaffected and penicillins are regarded as one of the safest and most efficacious class of antibiotics being used for bacterial infections.

The analysis of degradation products in commercial penicillins has two-fold importance; firstly in pharmacokinetic studies it is desirable to distinguish between the drug and any degradation products, secondly allergic reactions attributed to penicillin may frequently be caused by such compounds. Accordingly it is essential to be able to detect the presence of these compounds in the pharmaceutical compounds. HPLC methods have been widely employed for such analysis (Table 2) involving control of purity of pharmaceutical samples. In most of the cases studied C_{18} columns were used and UV detection method was preferred. Effect of gamma radiation on both, the combination of amoxycillin sodium–potassium clavulanate and individual components was studied by Valvo et al. [29]

3. Cephalosporin

Cephalosporins are β-lactam antibiotics, with the same fundamental structural requirements as

Table 4
HPLC seperation of tetracyclines

Name of compound	Stationary phase	Mobile phase	Ref.
Tetracycline antibiotics and their common contaminants	μ Bondapak C_{18}	0.05 M-KH_2PO_4 buffer of pH 2.5/MeCN (21:2)	[50]
Tetracycline and its degradation products in methanol	Apex I ODS silica	0.1 M-Oxalic acid of pH 2/MeOH/MeCN (17:2:1 to 19:9:12 in 25 min) or with 0.2 M-ammonium trifluoroacetate of pH 2.5/MeOH/MeCN (17:2:1 to 5:6:9 in 40 min)	[51]
Tetracycline hydrochloride	Alltima C_8	0.1 M-Ammonium dihydrogen phosphate buffer/DMF/0.1 M-ammonium oxalate (50:270:680)	[52]
Tetracycline and its major degradation products	Chromspher C_8	MeCN/0.01 M-oxalic acid of pH 2	[53]
Minocycline, tetracycline and chlortetracycline as aluminium complexes	Kromasil ODS C_{18}	Aqueous 10% MeCN containing 25 mM-lithium sulphate adjusted to pH 2.9 with H_2SO_4	[54]

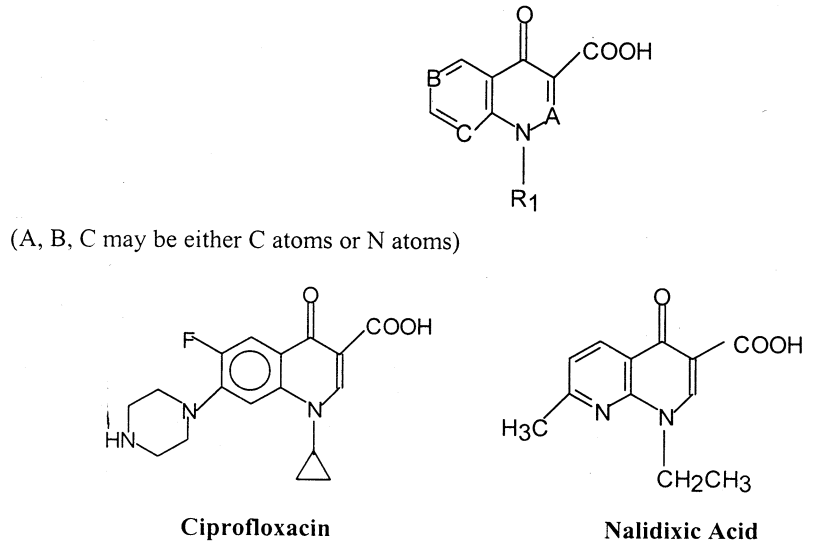


Fig. 4. Chemical structures of some important quinolones.

penicillin (Fig. 2). Heterocyclic ring fused to β-lactam ring is six membered (dihydrothiazine) in cephalosporins. The fused rings in β-lactams are not coplanar but folded along the C–N bond common to both rings; less markedly in cephalosporins than in penicillins. Cephalosporins are used for the treatment of infections caused by most gram-positive and -negative bacteria, especially *Escherichia coli*, *Proteus mirabilis* and klebsiella. As discussed in penicillin only cephalosporin C is found in nature isolated from cultures of fungi other i.e. semi-synthetic cephalosporins are derived from 7-amino-cephalosporanic acid, product obtained from cephalosporin C hydrolysis [33]. Literature suggests [34–48] (Table 3) use of C_{18} column for chromatographic analysis of this class of antibiotics. Carbanepem, a newly synthesized β-lactam antibiotic, was analysed for its degradation products by multistage liquid chromatography-electrospray mass spectrometry [38].

4. Tetracyclines

Tetracycline is an extremely important group of antibiotics having broad spectrum of activity against gram-positive and -negative bacteria, some large viruses, rickettsiae, spirochetes and mycoplasmas. They are also widely used as growth additives in animal feed. Chemically, tetracyclines contain an octahydronaphthacene ring skeleton, consisting of four fused rings (Fig. 3). Besides natural tetracyclines isolated from various strains of *streptomyces*, many derivatives (e.g. doxycycline, minocycline) have been prepared by their chemical conversion [49].

HPLC has been the prime analytical method employed in the analysis of tetracyclines during fermentation and isolation (Table 4). Presence of large number of polar functional groups make their analysis difficult as they tend to absorb strongly on silanol sites of support material, but use of reversed phase silica columns have solved the problem.

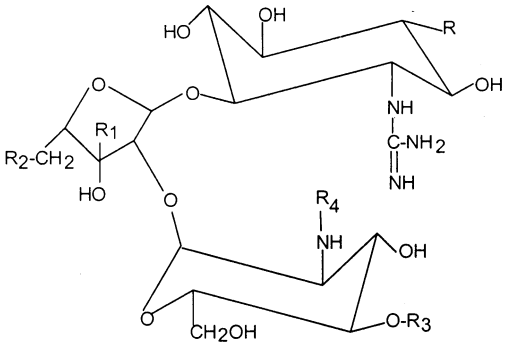
Denton and coworkers [51] have identified more than 14 degradation products of tetracycline in methanol within 6 months under normal storage conditions by HPLC, HPTLC and spectrophotometry. Post column derivatization of tetracycline and its major degradation products with magnesium acetate, boric acid and potassium hydroxide has been reported [53] followed by fluorescence detection.

5. Quinolone

Quinolone carboxylic acids are a class of totally synthetic antibacterial agents, which have the general structure as shown in Fig. 4. Carboxylic acid group present on 3-position, small alkyl or aryl group in 1-position and in the newer generation of quinolones a fluorine attached on 6-position (fluoroquinolones) and sometimes a nitrogen heterocycle attached to 7-position are responsible for antibacterial activity [55]. Initially, these compounds were applied in the treatment of urinary tract infections but now have a broad spectrum of activity in the treatment of both human and veterinary diseases. They do it by interfering with replication of bacterial DNA [56]. Isolation and analysis of quinolones by HPLC involved use of reversed phase column as discussed in Table 5. Results obtained with HPLC were compared with those of potentiometry [58] and titrimetry followed by UV spectrophotometry [64].

Table 5
HPLC separation of quinolones

Name of compound	Stationary phase	Mobile phase	Ref.
Ciprofloxacin, norfloxacin, ofloxacin and pipemidic acid	Lichrospher 100 RP-18	Various mixtures of 100 mM-tetrabutylammonium hydroxide in MeCN/H_2O (93:7) and 25 mM-H_3PO_4 in MeCN/H_2O (93:7) as mobile phase	[57]
Norfloxacin, enoxacin, fleroxacin, ofloxacin and flumequine	Shodex RS pak DS-631, a hydrophobic polygel packed column	30, 35 ,40 or 50% MeCN in buffers of pH 3–11, made from 25 mM-H_3PO_4 adjusted with NaOH	[58]
Pefloxacin, lomefloxacin and ofloxacin	μ-Bondapak C_{18}	H_2O/MeCN/TEA (850:150:1) adjusted to pH 3.5 with dilute H_3PO_4	[59]
Norfloxacin	Lichrosorb C8	Phosphate buffer of pH 3/MeCN (17:3)	[60]
Ciprofloxacin and tinidazole	C_{18}-Inertsil	0.1% Triethanolamine in water/MeCN (39:11) pH adjusted to 2.6 with 1% H_3PO_4	[61]
Sparfloxacin	CLC-ODS	5% Aqueous acetic acid/MeOH/MeCN (14:3:3)	[62]
Sarafloxacin	μ-Bondapak C_{18}	Aqueous 50% MeCN/MeOH/2 mM-H_3PO_4 adjusted to pH 3.5 with triethylamine (6:1:13)	[63]
Norfloxacin	Shim-pack ODS	0.1% H_3PO_4/MeOH (11:5) of pH 2.7 containing 1% triethylamine	[64]
Sparfloxacin	RP-select B	Triethylamine/MeCN/H_2O (0.1:10:40) adjusted to pH 2.6 with H_3PO_3	[65]
Ciprofloxacin	Lichrospher 100 RP-18	H_2O/MeCN/triethylamine (80:20:0.6), adjusted to pH 3.0 with orthophosphoric acid	[66]
Fluoroquinolone antibiotics viz. Ciprofloxacin, norfloxacin, ofloxacin, enoxacin	Shim-pack CLC-ODS	50 mM-Citric acid containing 0.01 M-ammonium acetate/MeOH (75:25) pH adjusted to 4.5 with triethylamine	[67]



R	R_1	R_2	R_3	R_4
$NHC(=NH)NH_2$	CHO	H	H	CH_3

Fig. 5. Chemical structure of streptomycin.

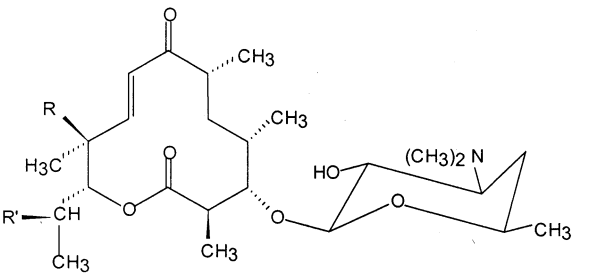


Fig. 6. Chemical structure of a 12-membered macrolide (Methymycin: R = OH, R′ = H).

6. Aminoglycosides

Antibiotics in this family characteristically contain two or more amino sugars joined via glycosidically linkage to deoxystreptamine or streptamine. Presence of several hydroxyl groups makes them hydrophilic in nature (Fig. 5). They are valuable in the treatment of serious infections caused by gram-negative bacteria. They inhibit the protein synthesis of microorganism resulting in a rapid concentration dependent bactericidal action [68].

HPLC and several other assay methods have been introduced for the analysis of aminoglycosides. Adams and coworkers [69] compared liquid chromatographic method with microbiological assay method for the determination of neomycins and proposed replacement of microbiological assay method by chromatographic method. HPLC methods involved use of reversed phase columns as mentioned in Table 6. Precolumn derivatization of analyte with phthaldehyde [73] was also found useful to make it more sensitive towards detection.

Table 6
HPLC separation of aminoglycosides

Name of compound	Stationary phase	Mobile phase	Ref.
Netilmicin sulfate	PL RP-S	35 g/l Na_2SO_4 containing 0.5 g/l sodium-1-octanesulfonate, 10 ml/l THF and 50 ml/l 0.2 M-phosphate buffer of pH 3	[70]
Tobramycin and related substances	Nova-Pak C_{18}	MeCN/buffer (containing Tris/H_2O/1N-H_2SO_4/MeCN) in the ratio 11:9	[71]
Amikacin	PLRP-S	Gradient of Na_2SO_4 in 10 mM-phosphate buffer of pH 3, containing 0.18% sodium octane sulfonate	[72]
Gentamicin sulphate	μ-Bondapak ODS	5.5 g Sodium heptane sulphonate monohydrate dissolved in MeOH/H_2O/anhydrous acetic acid (14:5:1)	[73]
Gentamicin (C_1, C_{1a}, C_2, C_{2a}, and C_{2b})	PLRP-S	Containing sodium sulphate (60 g/l), sodium 1-octane sulfonate (1.75 g/l), THF (8 ml/l) and 0.2 M-phosphate buffer of pH 3 (50 ml/l)	[74]
Dihydrostreptamycin sulphate	Base deactivated reverse phase silica gel	Aqueous solution containing 4 g/l of sodium sulphate, 1.5 g/l of sodium octanesulfonate, 100 ml/l of MeCN and 50 ml/l of 0.2 M-phosphate buffer	[75]

7. Macrolides

These agents are generally used to treat infection in the respiratory tract, skin and soft tissues and genital tract caused by gram-positive organisms, mycoplasma species and certain susceptible gram-negative and anaerobic bacteria. The generalized structure is a highly substituted monocyclic lactone (aglycone) to which is attached one or more saccharide units (amino or deoxy sugar) glycosidically linked to hydroxyl groups on either the aglycone or another saccharide. They may be divided into 12-, 14- and 16-membered aglycone ring macrolides (Fig. 6). Most of them are derived from various strains of streptomyces and serve as an alternative for patients exhibiting penicillin sensitivity. They inhibit growth of bacteria by inhibiting protein synthesis on ribosomes [76].

The literature suggests the use of ODS columns for the HPLC separation of macrolide antibiotics [77–91] (Table 7). An interlaboratory study of two HPLC methods involving different columns showed that reversed phase columns performed better than copolymer column [85]. Polyene macrolides, a class of antifungal antibiotics, form a subdivision of macrolide antibiotics containing a chromophore formed by a system of three to seven conjugated double bonds in macrolactone ring. Purification of polyene macrolide antibiotics is difficult because of presence of different polyenic and non-polyenic products [81,84,86].

Table 7
HPLC separation of macrolides

Name of compound	Stationary phase	Mobile phase	Ref.
Azithromycin	Ultrasphere ODS	0.05 M-Phosphate buffer/MeOH/MeCN (27:29:44) of pH 7.5	[77]
Acetylspiramycin	Hypersil ODS	MeCN/aqueous 0.1 M-ammonium acetate of pH 7.2 (3:2)	[78]
Erythromycin	PLRP-S	2-Methyl-2-propanol/MeCN/0.2 M-phosphate buffer of pH 11/H_2O (33:6:10:151)	[79]
Josamycin propionate	PLRP-S	MeCN/0.2 M-potassium phosphate buffer of pH 10/H_2O (13:5:7)	[80]
Amphotericin B	μ-Bondapak C_{18}	MeOH/MeCN/2.5 mM EDTA (10:7:4)	[81]
Medicamycin A in meleumycin	Ranin C_{18}	0.1 M Ammonium formate/MeCN (20:11) adjusted to pH 6 with 10% acetic acid	[82]
Azithromycin	μ-Bondapak C_{18}	67 mM-KH_2PO_4 buffer of pH 4.0/MeCN (7:3)	[83]
Amphotericin B in oil–water emulsion	Spherisorb C_{18}	MeOH/5 mM-EDTA	[84]
Tylosin	Method A-ODS silica gel Method B-styrene-divinyl benzene copolymer	MeCN/22.5% sodium perchlorate of pH 2.5 (2:3) THF/3.5% K_2HPO_4 solution of pH 9.0/H_2O (4:1:15)	[85]
Fosfomycine	Zorbax-ODS	Methanol/H_2O (1:1) of pH 3.18 containing 2 mM acetic acid and 10 μM-acridine	[86]
Macrolide and ketolide antibiotics	Pharmacia LKB Spherisorb ODS-2	MeCN/MeOH/0.1 M-ammonium acetate buffer of pH 7 (13:4:3)	[87]
Roxithromycin	Base-deactivated silica gel	MeCN/H_2O/ammonium dihydrogen phosphate (ADHP) optimized with 26% ADHP of pH 4.3	[88]
Roxithromycin	Kromasil C_{18}	MeCN/MeOH/0.5% ammonium acetate (5:3:3)	[89]
Erythromycin estolate	Nova-Pak C_{18}	20 mM ammonium acetate of pH 6.7/MeCN (2 : 3)	[90]
Erythromycin	Xterra RP C_{18}	2-Methyl propan-2-ol/propan-2-ol/0.2 M ammonium carbonate buffer/H_2O (1.5:1.5:1:16) and 2-methyl propan-2-ol/MeCN/0.2 M ammonium carbonate buffer/H_2O (15:3:5:77)	[91]

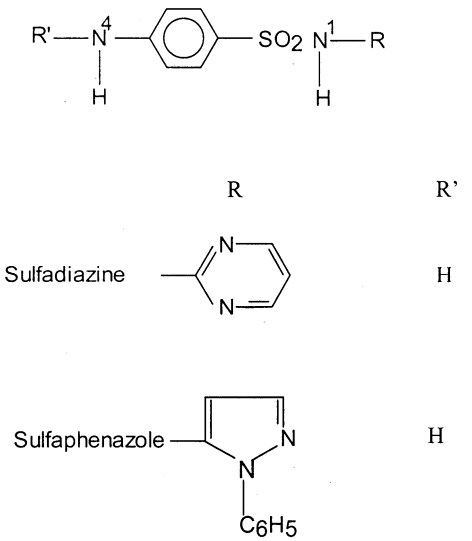


Fig. 7. Chemical structure of sulfonamides.

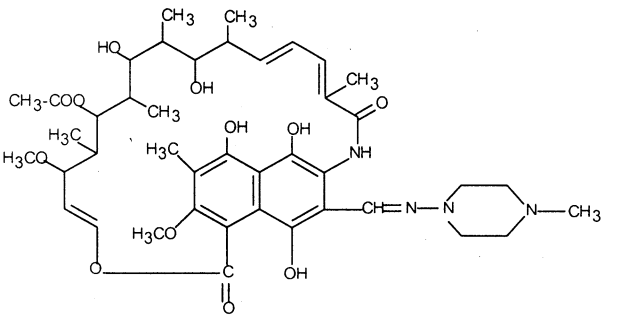


Fig. 8. Chemical structure of rifampin.

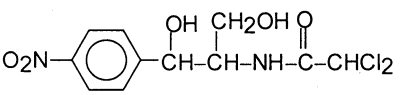


Fig. 9. Chemical structure of chloramphenicol.

8. Sulfonamides

These are first drugs effective against bacterial septicemias, but also effective in tissue infections due to streptococci and fungus related nocardia [92]. Nowadays they are widely used in farm animal feedstuff and fish cultures as veterinary drugs for prophylactic and therapeutic purposes. Sulfonamides inhibit growth of bacteria in-vivo only if the medium is free of inactivating substances, mainly peptones and *p*-aminobenzoic acid. Principal disadvantage of sulfonamide therapy is however emergence of drug resistant strains of bacteria, this has led to replacement of drug by

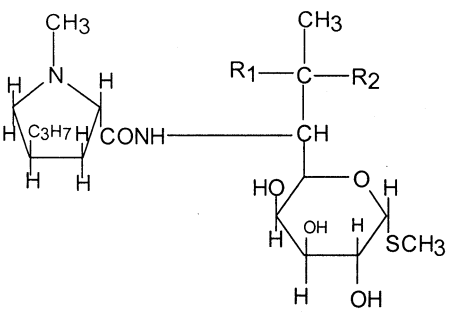


Fig. 10. Chemical structure of lincomycin ($R_1 = OH$; $R_2 = H$) and clindamycin ($R_1 = H$; $R_2 = Cl$).

Table 8
HPLC separation of sulfonamides

Name of compound	Stationary phase	Mobile phase	Ref.
Sulphadiazine, sulphamethoxazole and trimethoprim	YWG-$C_{18}H_{37}$	Phosphate buffer (100 ml, 50 mM KH_2PO_4 and 2 ml 50 mM-triethylamine with pH adjusted to 5.9 with H_3PO_4)/MeOH (4:1)	[93]
Sulphacetamide sodium and sulphanilamide	Spherisorb C_{18}	0.25% Acetic acid adjusted to pH 7 with ammonia H_2O/MeOH (93:7)	[94]
Sulphacetamide and its hydrolysis product	Nucleosil C_{18}	H_2O/MeOH/acetic acid (89:10:1) containing 0.2% sodium-hexane-1-sulfonate	[95]
10 Selected sulphonamides	RP column	Octanol–water	[96]
Sulfamethazine	C_{18}	MeCN/2% acetic acid (17:83)	[97]

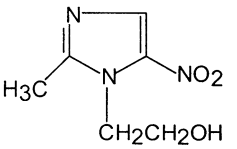


Fig. 11. Chemical structure of metronidazole.

penicillin and other semi-synthetic antibiotics to a large extent. In the third world countries where problems of storage and lack of medical personnel make appropriate use of antibiotics difficult, they are of great value.

They are derived from sulfanilamide (*p*-aminobenzenesulfonamide) and commonly known as sulfa drugs. Although several therapeutically active derivatives are usually substituted on the N^1 nitrogen; the N^4 position is generally unsubstituted (Fig. 7).

HPLC methods for the analysis of sulphonamides have been discussed in Table 8. However, use of multivariate and principal component analysis, for the chemometric study of retention data obtained from HPLC analysis of 12 sulphonamides has also been cited in literature [98].

9. Miscellaneous antibiotics

9.1. Rifamycin

Rifampin (Fig. 8) the most important compound of rifamycin group inhibits the growth of most gram-positive and some -negative microorganisms by inhibiting their RNA synthesis [99]. Rifampin on its own or in association with isoniazid is employed in treating tuberculosis.

Table 9
HPLC separation of miscellaneous antibiotics

Name of compound	Stationary phase	Mobile phase	Ref.
Rifamycin and its impurities	Lichrospher 100 RP-8	75 mM-KH_2PO_4/MeCN/MeOH (1:1:1)	[101]
Metronidazole and its hydrolysis product	Bio-Sil C_{18}-5S	Component A/MeCN (7:3) Component A: (6 mM-SDS/2.5 mM-H_3PO_4 of pH 3.5)/MeCN (9:1)	[102]
Rifampicin, isoniazid and pyrazinamide in tablets	Spherisorb CN	MeCN/0.01 M-sodium-1-heptane-sulphonate with pH adjusted to 2 (14:11)	[103]
Chloramphenicol	C_{18}	Aqueous 85% methanol	[104]
Lincomycin and related substances; lincomycin B and 7-epilincomycin	Base deactivated Supelcosil LC-ABZ C_{12-18}	2.25 % MeCN/2.72% KH_2PO_4 adjusted to a pH of 5 with 3.48% K_2HPO_4 and 0.067% methanesulfonic acid in H_2O	[105]
Rifampicin and its acid-induced degradation products	Nova-Pak C_{18}	0.02 M-Na_2HPO_4/MeCN (13:7) adjusted to pH 4.5 with H_3PO_4	[106]
Sulfur-containing antibiotics penicillins, cephalosporins and lincomycin	Phenomenex Luna C_8	Aqueous 90% MeOH, 0.5 M-sodium acetate buffer of pH 3.75, H_2O and aqueous 90% MeCN	[107]
Chloramphenicol	YWG-C_{18}	H_2O/MeOH/glacial acetic acid (550:450:1)	[108]
Clindamycin and related substances	Hypersil-ODS	MeCN/phosphate buffer (1.35% phosphoric acid adjusted to pH 6 with ammonium hydroxide)/H_2O (7:8:5)	[109]
Metronidazole and ciprofloxacin	Reversed phase	–	[110]
Aminosalicylic acid and isoniazid	Shimadzu CLC-ODS	0.05% SDS/MeOH (7:1; containing 0.2% sodium acetate)	[111]
Colistin	YMC-Pack Pro C-18	MeCN/Sodium sulphate (0.7%m/v)/phosphoric acid (6.8% v/v dilution of 85% m/m H_3PO_4)/H_2O (21.5:50:5:23.5)	[112]

9.2. Chloramphenicol

Chloramphenicol (Fig. 9) first employed in the late 1940s to treat a typhus epidemic in Bolivia [100], inhibits protein synthesis in bacteria and to a lesser extent in eukaryotic cells. It has an antimicrobial spectrum similar to that of tetracycline. Nowadays it is mainly applied in veterinary medicine because of its adverse reaction in humans.

9.3. Lincomycin and clindamycin

Lincomycin and its clinically more useful analogue, clindamycin (Fig. 10) are active against many gram-positive bacteria but are inactive against gram-negative species as they inhibit protein synthesis of former.

9.4. Metronidazole:

Metronidazole an antiprotozoal is important in the treatment of amoebic dysentery and trichomoniasis (Fig. 11). Details of HPLC analysis of rifamycin, chloramphenicol, lincomycin etc. have been tabulated (Table 9).

10. Conclusions

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 0.3–0.5 μg/ml. Sensitivity can be further enhanced by coupling it with fluoremetric, 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. Derivatization of sample may also improve its sensitivity towards detection as in case of aminoglycosides.

Hundreds of antibiotics and their semisynthetic analogues are being discovered every year owing to development of resistance in micro-organisms for previous ones. This emphasizes need for continuous search for modern analytical methods to study pharmaceutical as well as biological samples and thus HPLC can well serve the challenging field of unlimited possibilities.

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