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Review

Analysis of recent antimicrobial agents in human biological fluids by high-performance liquid chromatography

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Abstract

Our previous review on the liquid chromatographic (LC) analysis of anti-bacterial agents was published in 1990 in a special issue of the *Journal of Chromatography*. Eight years later, some new agents have been registered and numerous other are under clinical experiment. In spite of therapeutic problems encountered with certain bacterial pathogens, the development of novel drug candidates has slowed partially due to the need for identification of new bacterial targets and the cost of the research. The present overview updates the LC methods for the quantitations of recent antimicrobial agents (marketed and in clinical development) in human biological fluids. Consideration has been given to procedures permitting the determination of isomers and metabolites as well as methods regarding tissue extracts or liquid sampled from physiological sanctuaries. LC methods are available for the quantitation of almost all registered or investigated recent anti-infective drugs and some are applicable in routine practice. Nevertheless, few techniques have been validated for the determination in tissue extracts limiting the development of penetration studies.

Keywords: Reviews; Pharmaceutical analysis; Antibiotics; Antimicrobial agents

Contents

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1. Introduction

Our previous review on the liquid chromatographic (LC) analysis of antibacterial agents in biological fluids was published in 1990 in a special issue of the *Journal of Chromatography* [1]. It described the successive steps to be followed to develop a technique for the major antibacterial agents. Eight years later, some new products have been registered and numerous other are under experiment. It has to be stressed that in spite of therapeutic problems, e.g., resistance encountered in certain bacterial pathogens, the development of novel drug candidates has slowed [2–4]. This is partially due to the cost of the research and the need for identification of new bacterial targets. The development of recent antimicrobial agents has mainly focused on orally bioavailable broad spectrum drugs (fluoroquinolones, third generation cephalosporins), carbapenems and molecules active against Gram positive cocci. From a technical point of view, the last decade has been characterized by the introduction of capillary electrophoresis, a new analytical methodology that has been proved to be applicable to the determination of drugs in biological fluids (i.e., to pharmacokinetics) (reviewed in Ref. [5]).

The present overview updates the LC methods for the quantitation of recent antimicrobial agents (marketed and in clinical development) in human biological fluids. Consideration has been given to procedures permitting the determination of isomers and/or metabolites as well as methods regarding tissue extracts or liquids extracted from physiological sanctuaries. Besides the classical application of analytical methods (i.e., determination of kinetic parameters) there is a need for validated techniques permitting the conduct of tissue penetration studies as well as procedures for therapeutic drug monitoring adaptable in routine practice given the development of clinical pharmacokinetics/pharmacodynamics of antimicrobial agents. In addition, pharmacokinetics constitute a critical step to early evaluation of an antimicrobial agent because unsatisfactory kinetics e.g., poor absorption, are responsible for nearly all antiinfective termination, contrasting with other pharmacological classes [6]. Hence analytical procedures must be developed and kinetic properties determined as early as possible.

2. Methodological considerations

The information sources included computerised and manual data bases (Medline, Current Contents). The search concerns papers published in French and English in peer-reviewed publications after 1990. The papers dealing with the LC analysis of recently approved and investigational (phase I–III) antimicrobial agents (antibacterial, antifungal) in human biological fluids were selected. Articles referring to drugs used in veterinary science or cited in our previous review and analysis in pharmaceutical forms were excluded. Papers published before 1990 relating techniques for marketed products that were not included in our previous review were considered. The search was stopped in January 1998. The different LC techniques are presented in Table 1.

3. Penams

Aspoxicillin is a new parenteral broad spectrum penicillin deriving from amoxicillin and mainly investigated in Japan [7]. One procedure regarding the determination of aspoxicillin in broncho–alveolar lavage fluid has been reported [8]. To increase sensitivity, the authors used a photochemical reaction system, a method of detection that has received considerable interest since the early ’90s [9]. Following post-column photolysis, the degradation products were detected by electrochemistry, instead of the classical UV detection of the penicillins. Using this system, the authors obtained an impressive limit of detection (LOD) of 1 ng/ml [8].

4. Penems

Carbapenems antibiotics are considered to be among the most potent antibacterial agents (i.e., broad spectrum of activity and highly active against resistant pathogens). Meropenem (Smithkline Beecham) is the second approved carbapenem after imipenem which is combined with cilastatin. Unlike imipenem, meropenem exhibits a relative stability to hydrolysis by the proximal tubular brush border enzyme dihydropeptidase I (DHP-I) [10]. Hence the parenteral formulation of meropenem does not con-

Table 1
Liquid chromatographic methods for the determination of recent antimicrobial agents in human biological fluids

Chemical or pharmacological class	Drug	Biological fluid	Column	Mode of detection	LOD or LOQ (ng/ml)	Ref.
Penam	Aspoxicillin	Broncho–alveolar lavage fluid	C_{18}	Electrochemical after photolysis	1 (LOD)	[8]
Penem	Meropenem and metabolite	Plasma	C_{18}	UV 296 nm	60 (LOD)	[10]
		Urine	C_{18}	UV 220 nm	500 (LOD, metabolite)	[10]
	Meropenem	Plasma, urine	C_{18}	UV 296 nm	400 (LOD, plasma) 4000 (LOD, urine)	[11]
		Plasma, urine	C_{18}	UV 296 nm 313 nm	500 (LOQ) 1000 (LOQ)	[12]
		Serum	C_{18}	UV 298 nm	250 (LOQ)	[13]
	DA 1131	Plasma, urine	C_{18}	UV 300 nm	100 (LOQ, urine) 500 (LOD, plasma)	[14]
	FCE 22101	Plasma, urine	C_{18}	UV 320 nm	5 (LOD, plasma)	[15]
Cephem	Cefepime	Plasma, urine	C_{18}	UV 280 nm	2000 (LOD)	[16]
		Plasma	C_{18}	UV 260 nm		[17]
		Serum	C_{18}	UV 254 nm	500 (LOQ)	[18]
	Cefozopran	Serum, urine	C_{18}	UV 235 nm	620 (LOD, serum) 3500 (LOD, urine)	[19]
	Cefprozil	Plasma, urine	C_{18}	UV 280 nm	500 (LOQ)	[20]
	diastereoisomers	Plasma, tissue extract	C_{18}	UV 280 nm	100 (LOQ) 100 ng/g tissue (LOQ)	[21]
	Cefcanel	Plasma, urine	C_{8}	UV 254 nm	0.2 n*M* (LOQ, plasma) 2 n*M* (LOQ, urine)	[23]
	Cefetamet and prodrug	Plasma, urine	C_{18}	UV 265 nm	200 (LOQ, plasma) 500 (LOQ, prodrug) 20 000 (LOQ, urine)	[24]
	Cefetamet	Plasma	C_{18}	UV 265 nm	100 (LOD)	[25]
		tissue extract	C_{18}	UV 265 nm	300 (LOQ)	[26]
	Ceftibuten	Plasma, urine	C_{18}	UV 262 nm (plasma) UV 254 nm (urine)	500 (LOQ)	[27]
		Plasma, urine, Milk, Inflammatory fluid	C_{18}	UV 254 nm	100 (LOQ, plasma, fluid) 500 (LOQ, urine) 1000 (LOQ, urine)	[28]
		Plasma, urine	C_{18}	UV 254 nm	100 (LOQ, plasma) 500 (LOQ, urine)	[29]
	Ceftibuten and metabolite (Ceftibuten-trans)	Plasma	C_{18}	UV 254 nm	100 (LOQ) 200 (LOQ, metabolite)	[30]
		Plasma	C_{18}	UV 254 nm	200 (LOQ) 500 (LOQ, metabolite)	[31]
	Ceftibuten	Middle ear fluid	Double C_{18}	UV 254+263 nm	500 (LOQ)	[32]

(Cont.)

Table 1. Continued

Chemical or pharmacological class	Drug	Biological fluid	Column	Mode of detection	LOD or LOQ (ng/ml)	Ref.
Aminoglycosides	Isepamicin	Plasma	C_{18}	Fluorescence after post-column derivatization ex: 338 nm, em: 450 nm	100 (LOQ)	[33]
		Plasma, urine dialysate	C_{18}	Fluorescence after post-column derivatization ex: 338 nm, em: 418 nm	100 (LOQ, plasma) 50 (LOQ, urine dialysate)	[34]
		Plasma	C_{18}	UV 265 nm after pre-column derivatization	500 (LOQ)	[35]
Fluoroquinolones	Trovafloxacin	Serum, urine	C_{18}	UV 275 nm	100 (LOQ)	[37]
	Balofloxacin	Plasma, urine	C_8	Fluorescence ex: 295 nm, em: 500 nm	10 (LOD)	[40]
	Ibafloxacin	Plasma, urine	PRP	UV 313 nm	100 (LOQ)	[42]
	Grepafloxacin	Plasma, urine	C_{18}	Fluorescence ex: 285 nm, em: 448 nm	25 (LOD, plasma) 100 (LOD, urine)	[40]
	Clinafloxacin	Serum	C_{18}	UV 275 nm	100 (LOD)	[44]
	Rufloxacin and metabolites	Plasma, urine, bile	Polystyrene-di-vinyl-benzene	Fluorescence ex: 350 nm, em: 510 nm	10 (LOD)	[46]
		Plasma, urine	C_{18}	UV 296 nm	500 (LOD)	[47]
	Rufloxacin	Plasma, urine	PRP 1	Fluorescence ex: 294 nm, em: 475 nm	500 (LOD, plasma) 300 (LOD, urine)	[48]
Macrolides	Rokitamycin and metabolites	Plasma	C_{18}	Fluorescence after pre-column derivatization ex: 352 nm em: 537 nm	200 (LOD)	[49]
	Metabolites of miocamycin	Plasma	C_{18}	UV 229 nm		[51]
Agents against Gram positive pathogens	Quinupristin and metabolites	Plasma	C_{18}	Fluorescence ex: 360 nm, em: 410 nm	25 (LOQ, quinupristin) 10 (LOQ metabolites)	[55]
	Dalfopristin and metabolites	Plasma	C_{18}	UV 235 nm	25 (LOQ)	[55]
	Rifapentine	Serum	C_{18}	UV 336 nm	5 (LOQ)	[56]
Azoles	Itraconazole	Plasma, tissue homogenate	C_{18}	UV 254 nm	1 (LOD, plasma)	[59]
	Itraconazole	Plasma	C_{18}	Fluorescence ex: 265 nm, em: 355 nm	20 (LOD)	[60]
	Itraconazole	Plasma, tissue homogenate	C_{18}	UV 263 nm	5 (LOD, plasma) 10 ng/g (LOD, tissue)	[61]
	Itraconazole and metabolites	Plasma	C_{18}	UV 263 nm	35 (LOD, metabolite) 50 (LOD)	[62]
	Itraconazole	Serum	C_{18}	UV 260 nm	313 (LOD)	[63]
	Itraconazole and metabolites	Serum	C_{18}	UV 263 nm	2.5 (LOQ) 5 (LOQ, metabolite)	[64]
	Genaconazole	Serum, urine	C_{18}	UV 208 nm	200 (LOQ, serum) 500 (LOQ, urine)	[65]
	Genaconazole enantiomers	Serum	β-Cyclo-dextrin	UV 205 nm	200 (LOQ)	[66]

LOD=Limit of detection, LOQ=limit of quantitation, ex=excitation, em=emission.

tain cilastatin, an inhibitor of DHP-I. Regards the analysis of meropenem, the serum specimen does not need pretreatment (i.e., stabilisation with ethylene glycol and morpholinoisopropanesulfonic acid) when compared with imipenem. Meropenem is assayed by reversed-phase chromatography with UV detection (296 nm) [10–13]. The analysis is applicable in routine practice [13]. Much pharmacokinetic data have been already published and have revealed that meropenem is mainly eliminated unchanged by the kidneys [10]. Meropenem undergoes metabolism giving a microbiologically inactive β-lactam ring-opened product [10].

DA 1131 (Dong-A Pharmaceutical) is a carbapenem investigated in South Korea [14]. DA 1131 has been shown to be unstable in human plasma at room temperature [14]. Plasma samples should be kept at −70°C until analysis. Development of this compound is in a preclinical phase and pharmacokinetic data are available in animal models. One technique has been published for the determination of DA-1131 in human spiked plasma and urine specimen [14].

The broad spectrum penem FCE 22101 (Farmitalia) is the active moiety resulting from the intestinal hydrolysis of the oral prodrug FCE 22891 [15]. Preliminary pharmacokinetic studies with FCE 22891 have shown that the prodrug was entirely hydrolysed before reaching the systemic circulation e.g., no prodrug has been detected in human plasma and urine specimen [15]. Matsuoka et al. [15] proposed a LC method for the determination of the sole active form FCE 22101 in biological fluids using reversed-phase LC and UV detection (320 nm).

5. Cephems

Cefepime (Bristol Myers Squibb) is a recently approved parenteral broad spectrum cephalosporin assimilated as a so called fourth generation family member. Cefepime chemically differs from the third generation cephalosporins by a quaternary nitrogen substitution at the 3 position of the cephem nucleus making it a zwitterion. Cefepime is unstable at room temperature (100% loss after 24 h) necessitating immediate providing to the laboratory after drawing blood and a rapid analytical method. In case of delayed dosage, the serum samples can be kept at −80°C. Hence, given its instability at 20°C and the length of the procedure (24 h), the microbiological assay is inadequate. LC is the recommended method for cefepime analysis [16–18]. Cefepime is easily and rapidly analyzed in plasma by reversed-phase chromatography and UV detection set at 254 nm [18]. This procedure is applied in our laboratory for the therapeutic monitoring in severely ill patients (run time less than 3 min). Cefepime is mainly eliminated by the kidneys as an unchanged drug.

Cefozopran is a parenteral broad spectrum cephalosporin developed by Takeda with potent in-vitro activity against a variety of clinical pathogens. Little is known about the analysis of this drug. One paper described a reversed-phase chromatographic procedure for the determination in serum and urine [19]. Kinetic data have revealed that no metabolites have been detected by HPLC.

As stated before, most of cephalosporin drug research has focused on broad spectrum oral forms. Former approved oral cephalosporins do not display the same spectrum of activity of parenteral analogs (in terms of ''generation'') partially because of unsatisfactory kinetic profiles (low bioavailability, low tissue penetration). Hence they are mainly used in the management of infections in outpatients.

Bristol Myers Squibb is developing cefprozil, an oral cephalosporin consisting of a mixture of *cis*- and *trans*-isomers in a ratio 9:1 [20]. Both isomers exhibit an antibacterial activity [20]. Simultaneous quantification of the isomers in human biological fluids has been reported by Shyu et al. [20], using reversed-phase C_{18} column and UV detection set at 280 nm. Early kinetic data have revealed that both isomers display similar disposition and that no biotransformation occurred [20]. The same method was adapted for the measurement of cefprozil isomers in tonsillar and adenoidal tissues [21].

Cefcanel daloxate hydrochloride (Astra) is a prodrug for oral use. It is a double cephem ester of cefcanel [22] which is the active entity obtained after removal of the alanine ester in the intestinal lumen and then hydrolysis of the monoester in the gut wall. Preliminary kinetic studies obtained in animals and in man have indicated that the prodrug is not detectable in blood [22]. In addition, cefcanel is mainly eliminated by the renal route (90% recovery

after i.v. administration) [22]. Lanbeck-Vallen and Carlqvist [23] reported a procedure for the determination of cefcanel in human plasma and urine, by reversed-phase chromatography using coupled columns to achieve selectivity from endogenous compounds with UV detection.

Cefetamet pivoxyl (Roche) is an oral inactive prodrug which is hydrolysed in cefetamet, the microbiologically active metabolite. Cefetamet pivoxyl has been shown to be unstable in spiked serum and hence must be stabilised with citric acid and sodium fluoride [24]. Wyss and Bucheli [24] described a technique permitting the separation and the quantitation of the prodrug and the active metabolite by reversed-phase chromatography and UV detection (265 nm). Further pharmacokinetic investigations have shown that the prodrug was not detectable in the systemic circulation, as expected. Cefetamet is mainly excreted unchanged in urine.

Ceftibuten is an extended-spectrum oral cephalosporin (assimilated as a third generation) licensed by Schering Plough [31]. Ceftibuten is a *cis*-isomer that undergoes metabolic inversion in a *trans*-compound [31]. The *trans*-isomer exhibits about one-eighth the antibacterial activity of ceftibuten and is present in low amounts in plasma [31]. The urinary excretion represents less than 10% of the administered dose. Various techniques have been published [27–32] and some describe the separation and the quantitation of both isomers in biological fluids [30,31]. Reversed-phase chromatography is used with C_{18} stationary phases and UV detection set at 254 nm. The limit of quantification (LOQ) for *trans*-ceftibuten is twice that of the parent drug [30].

6. Aminoglycosides

Aminoglycosides are poor chromophoric drugs that necessitate derivatization to make them detectable by UV or fluorescence. It has to be noted that HPLC is not the primary methodology for the analysis of aminoglycosides in biological fluids. Fluorescence polarisation immunoassay (FPIA) is routinely performed for therapeutic monitoring.

Isepamicin is a recently approved semi-synthetic aminoglycoside, deriving from gentamicin B and licensed by Schering Plough. Various procedures for the measurement of isepamicin concentrations in biological fluids have been published [33–35], including a FPIA technique [36]. Regarding LC, the separations are performed on C_{18} columns and detection in UV or fluorescence after derivatization. The LOQ is lower when obtained with fluorescence detection. Since aminoglycosides do not undergo biotransformation, FPIA appears suitable except with uremic, lipemic or icteric samples.

7. Fluoroquinolones

Aimed at the treatment of systemic bacterial diseases, fluoroquinolones continue to generate great interest. Indeed, numerous broad spectrum fluoroquinolones are in preclinical or clinical development. The search of new analogues has focused on molecules with enhanced activity against Gram positive and anaerobic bacteria.

Trovafloxacin (Pfizer) is about to be approved in Europe, Japan, USA and will be available in both oral and parenteral formulations. Trovafloxacin is assayed in serum and urine by reversed-phase chromatography and UV detection [37]. The metabolic fate of C_{14}-labelled trovafloxacin has recently been investigated in humans and has revealed the presence of three metabolites (after characterisation by mass spectrometry) in serum and urine [38]. To our knowledge no chromatographic procedure (i.e., non radioactive) has been reported enabling the separation and the quantitation of trovafloxacin and its metabolites. The possible in vitro antimicrobial activation of the metabolites is unknown.

Balofloxacin (Chugai) is a 8-methoxy fluoroquinolone under experiment in Japan and South Korea. The introduction of the methoxy group is believed to enhance the bactericidal activity against fluoroquinolone resistant staphylococci [39]. Little information is available about this oral quinolone. Balofloxacin is analysed in biological fluids with reversed-phase LC and fluorescence detection [40]. A technique describing the determination of balofloxacin and its demethyl-metabolite in various rat fluids has been reported [41]. In humans, balofloxacin is largely excreted by the kidneys. The existence of potential metabolites has not been reported.

Grepafloxacin is a oral quinolone developed by

Glaxo Wellcome and that is about to be registered. Grepafloxacin concentrations are determined in biological fluids by reversed-phase LC and fluorescence detection [40]. Grepafloxacin is mainly eliminated through hepatic biotransformation. The metabolic fate remains unpublished as well as analytical procedures for the quantitation of the metabolites.

Very little data are available for ibafloxacin, an oral fluoroquinolone under development by 3M Laboratories. Miller et al. [42] described a reversed-phase chromatographic procedure with UV detection for the determination of ibafloxacin in human plasma and urine.

Parke Davis is experimenting clinafloxacin in a oral formulation. Pharmacokinetic data have shown that half of the dose is eliminated unchanged in the urine [43]. The existence of potential metabolites has not been reported. One paper described the determination of clinafloxacin in human serum [44].

Rufloxacin (Mediolanum) is one of the most documented investigational fluoroquinolones. Oral rufloxacin pharmacokinetics have been well characterised and revealed a partial urinary recovery (25% of the ingested dose), a low biotransformation and a long half-life (30 h) [45] permitting one daily administration. Rufloxacin and its *N*-demethyl compound (a minor metabolite) have been assayed in various biological fluids by reversed-phase LC (polymeric packing) and fluorescence detection [46–48].

8. Macrolides

Since the introduction of clarithromycin, dirithromycin and the azalide azithromycin, the macrolide drug clinical development has mainly focused on ketolides, a new class of 14-membered-ring macrolides derivating from erythromycin A and characterised by a 3-keto function in place of the L-cladinose sugar. These molecules display increased stability in acid medium and enhanced in vitro activity. Some are in preclinical or clinical development but to our knowledge no full paper concerning their analysis in human fluids has been published.

Rokitamycin (Pierre Fabre Médicament), is a semi synthetic 16-membered macrolide that is biotransformed by esterases in two active metabolites called leucomycin A_7 and leucomycin V [49]. Tod et al. [49] described a method for the determination of the parent drug and the two metabolites in human plasma. Since rokitamycin may be degraded by plasma esterases, the specimen must be extracted immediately or kept frozen at −80°C with an esterase inhibitor. The procedure consisted in reversed-phase LC with fluorescence detection after pre-column derivatization [49]. This mode of detection was investigated to enhance selectivity when compared with UV detection.

Miocamycin or ponsinomycin (Menarini) is an oral administered 16-membered macrolide derived from midecamycin and approved in some western countries [50]. Preliminary pharmacokinetic studies have been conducted using bioassays. It was later shown that miocamycin was intensively and totally biotransformed. In fact, intact parent compound could not been detected in plasma samples by LC. Although miocamycin displays an antimicrobial activity, it can be considered as a bioprecursor. The three major metabolites Mb_{12}, Mb_6, Mb_{9a} possess antibacterial activity and contribute to the overall antiinfective effect. None of the metabolites is as potent as the parent drug. One paper [51] related an analytical method for the determination of these three metabolites in plasma. The technique using reversed-phase LC with UV detection is partially described.

9. New agents against Gram-positive pathogens

Research for new compounds against Gram-positive pathogens is currently highly active since vancomycin resistant strains of *Enterococcus* spp. and more recently *Staphylococcus* spp., including *S. aureus* have emerged [52,53].

One of the most promising investigational agents is a parenteral semi-synthetic streptogramin RP 59500 developed by Rhône Poulenc. It is a 30:70 mixture of quinupristin, a derivative of pristinamycin IA and dalfopristin derivating from pristinamycin IIB. Both compounds demonstrate synergistic antibacterial activity against a wide variety of Gram-positive cocci. Little information is available on the analytical methods and hence on the kinetic properties of this streptogramin. Quinupristin is significantly excreted unchanged (when determined by

radioactivity) whereas dalfopristin is extensively biotransformed, the main metabolite being the microbiologically active pristinamycin IIA [54]. The main source of biotransformation seems to result from the instability of the streptogramin mixture at physiological pH [54]. Hence, quinupristin is converted into active cystine and gluthatione conjugates, dalfopristin in pristinamycin IIA itself unstable. Pharmacokinetic studies imply the stabilization of the components in biological fluids samples by means of acidification at pH 3–4. Rhône Poulenc Rorer have developed a LC procedure for the determination of quinupristin, the cystine and glutathione conjugates as well as dalfopristin and pristinamycin IIA in acidified human plasma [55]. The separation is performed on a C_{18} column and detection in fluorescence for quinupristin and its two metabolites and UV for dalfopristin and pristinamycin IIA.

Rifapentine is a rifamycin derivative exhibiting antituberculous properties as well as activity against *S. aureus*. A procedure using a C_{18} column and UV detection has been described for the determination in human serum [56].

10. Azoles

Azoles, and particularly triazoles, represent an important class of antifungal agents. Itraconazole (Janssen) is a recently approved triazole for the treatment of systemic fungal infections. Given its lipophilicity, it cannot be currently given parenterally and is only available in an oral form. Itraconazole is extensively biotransformed by various pathways in a very large number of metabolites. Its major metabolites in humans is hydroxy itraconazole the levels of which in plasma at steady state are twice those of the parent drug. In addition, it displays an in-vitro antifungal activity similar to that of the parent drug [57]. Therapeutic drug monitoring has become useful in patients with variable and/or poor drug absorption (i.e., immunocompromised) necessitating simple and rapid techniques both quantifying itraconazole and the active hydroxylated product in serum [58]. Numerous procedures have been published using reversed-phase (C_{18}) chromatography and UV or fluorescence detection and some are appropriate for routine analysis [59,64]. Two papers [59,61] reported the determination of the parent compound in tissue extracts.

Genaconazole is a triazole currently under experiment by Schering Plough, given orally and topically. It is a racemate of 50% *RR* active and 50% *SS* inactive. HPLC techniques have been developed for the determination of the racemate and the enantiomers in serum and urine [65,66]. The chiral procedure used a β-cyclodextrin column for the separation of the isomers [66]. Preliminary pharmacokinetic data have shown that both enantiomers exhibit similar kinetic behaviour. In addition, no genaconazole metabolite have been detected in biological fluids [66].

11. Conclusions

LC analytical methods are available (i.e., published) for almost all registered or investigated recent antimicrobial agents and some are applicable in routine practice. Nevertheless, very few techniques have been validated for the determination in tissue extracts or liquids issued from physiological sanctuaries limiting the development of penetration studies. Some have been performed but using the non specific microbiological assay.

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