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Review

Recent advances in the analysis of antibiotics by capillary electrophoresis

In this review, the main aspects related to the separation of different groups of antibiotics by CE as well as the different applications reported in the literature from the beginning 2003 till May 2005 will be provided to the readers. Firstly, the experimental conditions employed to achieve the analysis of antibiotics by CE are given. Then, the main applications performed in the pharmaceutical, clinical, food, and environmental fields have been reviewed making emphasis on sample preparation requirements needed in each case. Finally, the main conclusions and future prospects in this field are presented.

Keywords: Antibiotics / Capillary electrophoresis / Review
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1 Introduction

The term "antibiotic" refers to a very diverse range of chemical substances (produced from bacteria or fungi (natural way) or in a semisynthetic or synthetic way) that possess antibacterial activity, that is, that kill or inhibit the growth of microorganisms. They are used in human and animal medicine to prevent and treat diseases (European Medicines Agency, http://www.emea.eu.int/; checked on May 2005) [1].

Although HPLC is mainly used for the analysis of antibiotics by separation techniques, CE is being increasingly employed due to its favorable characteristics (high efficiency, large flexibility, and low consumption of samples and reagents). In addition, CE is being used in routine analysis because it allows obtaining appropriate analytical characteristics and good quantitative results. The analysis of antibiotics by CE is mainly included in two different working modes: (i) CZE where a separation buffer without or with additives is used for the separation of ionic or ionogenic antibiotics based on their different electrophoretic mobilities, and (ii) MEKC where a micellar system (surfactant at a concentration higher than its CMC) is added to the separation buffer to perform the separation of neutral and/or ionic or ionogenic antibiotics based on the generation of a pseudostationary phase in which analyte partition takes place. Although much less used, CEC and nonaqueous CE (NACE) have also been used for the analysis of antibiotics [2].

In order to provide to the readers an updated view of the separation conditions as well as the different applications reported in the analysis of antibiotics using CE as separation technique, this review covers the literature dealing on the analysis of antibiotics by CE from the beginning of 2003 till May 2005. Literature published before this date on this subject has already been reviewed by Flurer [2–5]. The experimental conditions employed to achieve the analysis of antibiotics by CE are first presented in this article. Section 3 of this work describes the main applications performed in the pharmaceutical, clinical, food, and environmental fields making emphasis on sample preparation requirements needed in each case. Finally, the main conclusions and future prospects in this field are presented.

2 Analysis of antibiotics by CE

Table 1 summarizes the antibiotics analyzed during the last 2.5 years using CE as the separation technique. They have been classified in different antibiotic groups indicating their molecular formula, formula weight, CAS number (number assigned by the Chemical Abstract Service (CAS) to identify a specific chemical compound), and structure. In addition to the CE separation conditions, the

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Abbreviations: LLE, liquid–liquid extraction; **OPA**, 1,2-phthalic dicarboxaldehyde; **SLE**, solid–liquid extraction

Table 1. Group, substance, molecular formula, formula weight, CAS number, structure, CE separation conditions, detection system, and LODs of the antibiotics included in this review

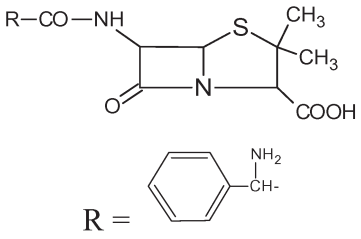
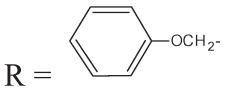
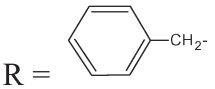
Group of antibiotic	Substance	Molecular formula (formula weight)[a)]	CAS number[b)]	Structure	CE separation conditions	Detection (LOD)	Reference
β-Lactam antibiotics							
	Ampicillin	$C_{16}H_{19}N_3O_4S$ (349.40)	69-53-4		40 mM phosphate-borate (pH 7.5) + 75 mM SDS (capillary 50 cm (57 cm) × 50 μm ID; 23–30 kV; 25°C)	UV-200 nm (0.1%)	[6]
					40 mM borate (pH 8.5) + 100 mM SDS (capillary 50 cm (57 cm) × 75 μm ID; 10 kV; 20°C)	UV-210 nm (0.2 μg/mL)	[7]
					20 mM borate (pH 8) + 60 mM SDS (capillary 55.5 cm (64 cm) × 75 μm ID; 15 kV; 25°C)	UV-210 nm (0.015 μg/mL)	[8]
	Penicillin V	$C_{16}H_{17}N_2O_5SK$ (388.48)	132-98-9		40 mM borate (pH 8.5) + 100 mM SDS (capillary 50 cm (57 cm) × 75 μm ID; 10 kV; 20°C)	UV-210 nm (0.15 μg/mL)	[7]
					20 mM borate (pH 8) + 60 mM SDS (capillary 55.5 cm (64 cm) × 75 μm ID; 15 kV; 25°C)	UV-210 nm (0.005 μg/mL)	[8]
	Penicillin G	$C_{16}H_{17}KN_2O_4S$ (372.48)	113-98-4		40 mM borate (pH 8.5) + 100 mM SDS (capillary 50 cm (57 cm) × 75 μm ID; 10 kV; 20°C)	UV-210 nm (0.3 μg/mL)	[7]
					10 g/L phosphate-borate (pH 8.7) + 14.4 g/L SDS (capillary 52 cm (60 cm) × 75 μm ID; 18 kV; 25°C)	UV-214 nm (1 μg/mL)	[9]
					80 mM borate (pH 8.0) (capillary 60 cm × 75 μm ID; 15 kV; 35°C)	UV-185 nm (3.5 μg/mL)	[10]
					20 mM borate (pH 8) + 60 mM SDS (capillary 55.5 cm (64 cm) × 75 μm ID; 15 kV; 25°C)	UV-210 nm (0.005 μg/mL)	[8]

Table 1. Continued

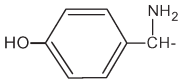
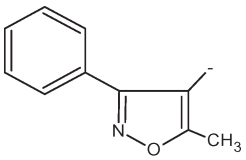
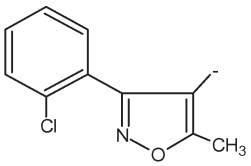
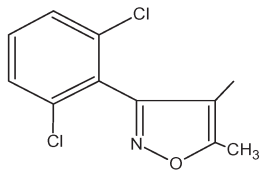
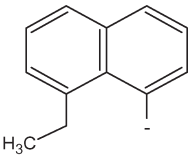
Group of antibiotic	Substance	Molecular formula (formula weight)[a)]	CAS number[b)]	Structure	CE separation conditions	Detection (LOD)	Reference
	Amoxicillin	$C_{16}H_{19}N_3O_5S$ (365.40)	26787-78-0	R = 	5–25 mM phosphate-borate (capillary 47–77 cm × 75–100 μm ID; 25 kV; 25°C)	UV-200 nm (0.015 μg/mL)	[11]
					40 mM borate (pH 8.5) + 100 mM SDS (capillary 50 cm (57 cm) × 75 μm ID; 10 kV; 20°C)	UV-210 nm (0.3 μg/mL)	[7]
					20 mM borate (pH 8) + 60 mM SDS (capillary 55.5 cm (64 cm) × 75 μm ID; 15 kV; 25°C)	UV-210 nm (0.025 μg/mL)	[8]
	Oxacillin	$C_{19}H_{18}N_3NaO_5S \cdot H_2O$ (441.43)	7240-38-2	R = 	40 mM borate (pH 8.5) + 100 mM SDS (capillary 50 cm (57 cm) × 75 μm ID; 10 kV; 20°C)	UV-210 nm (0.2 μg/mL)	[7]
					20 mM borate (pH 8) + 60 mM SDS (capillary 55.5 cm (64 cm) × 75 μm ID; 15 kV; 25°C)	UV-210 nm (0.005 μg/mL)	[8]
	Cloxacillin	$C_{19}H_{17}ClN_3NaO_5S$ (457.86)	642-78-4	R = 	40 mM borate (pH 8.5) + 100 mM SDS (capillary 50 cm (57 cm) × 75 μm ID; 10 kV; 20°C)	UV-210 nm (0.2 μg/mL)	[7]
					20 mM borate (pH 8) + 60 mM SDS (capillary 55.5 cm (64 cm) × 75 μm ID; 15 kV; 25°C)	UV-210 nm (0.005 μg/mL)	[8]
	Dicloxacillin	$C_{19}H_{16}Cl_2N_3O_5SNa \cdot H_2O$ (492.31)	13412-64-1	R = 	20 mM borate (pH 8) + 60 mM SDS (capillary 55.5 cm (64 cm) × 75 μm ID; 15 kV; 25°C)	UV-210 nm (0.005 μg/mL)	[8]
	Nafcillin	–	–	R = 	20 mM borate (pH 8) + 60 mM SDS (capillary 55.5 cm (64 cm) × 75 μm ID; 15 kV; 25°C)	UV-210 nm (0.015 μg/mL)	[8]

Table 1. Continued

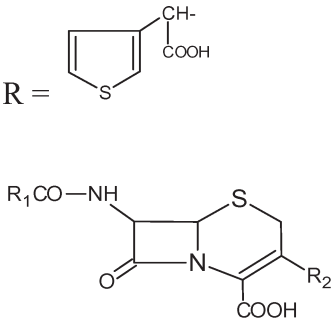
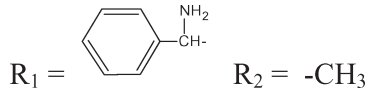
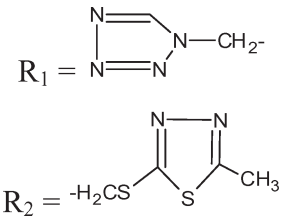
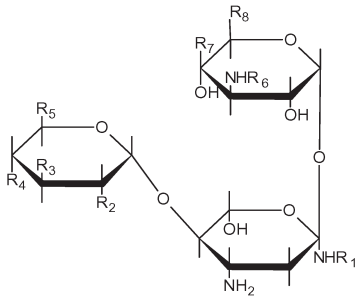
Group of antibiotic	Substance	Molecular formula (formula weight)[a)]	CAS number[b)]	Structure	CE separation conditions	Detection (LOD)	Reference
	Ticarcillin	$C_{15}H_{14}N_2Na_2O_6S_2$ (428.39)	4697-14-7		20 mM phosphate-borate (pH 8.66) + 1.44% SDS (capillary 60 cm × 75 μm ID; 18 kV; 25°C)	UV-214 nm (1.5 μg/mL)	[12]
	Cephalexin	$C_{16}H_{17}N_3O_4S \cdot xH_2O$ (347.39)	15686-71-2		20 mM borate (pH 9.23) + 20 mM SDS + 1% Brij 35 (capillary 28 cm (50 cm) × 75 μm ID; 15 kV; 30°C)	UV-210 nm (2 μg/mL)	[13]
	Cefazolin	$C_{14}H_{13}N_8NaO_4S_3$ (476.49)	27164-46-1		25 mM phosphate (pH 6.8) (capillary 40 cm (48.5 cm) × 50 μm ID; 25 kV; 25°C)	UV-270 nm	[14]
Aminoglycoside antibiotics							
	Kanamycin B	$C_{18}H_{37}N_5O_{10}$ (581.59)	29701-07-3	R_1 = –H, R_2 = $–NH_2$, R_3 = –OH, R_4 = –OH, R_5 = $–CH_2NH_2$, R_6 = –H, R_7 = –H, R_8 = $–CH_2OH$	30 mM borate (pH 10.0) + 16% methanol (capillary 31.5 cm (40 cm) × 50 μm ID; 15 kV; 20°C)	UV-335 nm (OPA derivative; ~0.6 μg/mL)	[17]
	Amikacin	–	149022-22-0	R_1 = $–COCH(CH_3)_2CH_2NH_2$, R_2 = –OH, R_3 = –OH, R_4 = –OH, R_5 = $–CH_2NH_2$, R_6 = –H, R_7 = –H, R_8 = $–CH_2OH$	100 mM borate (pH 10.0) + 20 mM deoxycholate + 15 mM β-CD (capillary 24.5 cm × 50 μm ID; 12 kV; 25°C)	UV-340 nm (OPA derivative)	[15]

Table 1. Continued

Group of antibiotic	Substance	Molecular formula (formula weight)[a)]	CAS number[b)]	Structure	CE separation conditions	Detection (LOD)	Reference
	Tobramycin	$C_{18}H_{37}N_5O_9$ (467.51)	32986-56-4	$R_1 = -H$, $R_2 = -NH_2$, $R_3 = -H$, $R_4 = -OH$, $R_5 = -CH_2NH_2$, $R_6 = -H$, $R_7 = -H$, $R_8 = -CH_2OH$	100 mM borate (pH 10.0) + 20 mM deoxycholate + 15 mM β-CD (capillary 24.5 cm × 50 μm ID; 12 kV; 25°C)	UV-340 nm (OPA derivative)	[15]
	Gentamicin (C_1, C_{1a}, C_{2a}, C_2)	–	1405-41-0	$R_1 = -H$, $R_2 = -NH_2$, $R_3 = -H$, $R_4 = -H$, $R_5 = -CH(NH_2)CH_3$, $R_6 = -CH_3$, $R_7 = -CH_3$, $R_8 = -H$	100 mM borate (pH 10.0) + 20 mM deoxycholate + 15 mM β-CD (capillary 24.5 cm × 50 μm ID; 12 kV; 25°C)	UV-340 nm (OPA derivative)	[15, 16]
					1 mM citrate (pH 3.5) + 0.2 mM CTAB (capillary 53 cm × 50 μm ID; 15 kV; 25°C)	Potential gradient detection (~9 μg/mL)	[18]
					60 mM CHES (pH 9.5) + 31.6% methanol (capillary 40 cm (50.2 cm) × 75 μm ID; 23 kV; 20°C)	UV-230 nm (~0.0001 μg/L)	[25]
	Sisomicin	$2C_{19}H_{37}N_5O_7 \cdot 5H_2O_4S$ (1385.45)	53179-09-2	$R_1 = -H$, $R_2 = -NH_2$, $R_3 = -H$, $R_4 = -H$, $R_5 = -CH_2NH_2$, $R_6 = -CH_3$, $R_7 = -CH_3$, $R_8 = -H$	100 mM borate (pH 10.0) + 20 mM deoxycholate + 15 mM β-CD (capillary 24.5 cm × 50 μm ID; 12 kV; 25°C)	UV-340 nm (OPA derivative)	[15, 16]
	Netilmicin	$C_{21}H_{41}N_5O_7 \cdot 2.5H_2O_4S$ (720.78)	56391-57-2	$R_1 = -C_2H_5$, $R_2 = -NH_2$, $R_3 = -H$, $R_4 = -H$, $R_5 = -CH_2NH_2$, $R_6 = -CH_3$, $R_7 = -CH_3$, $R_8 = -H$	100 mM borate (pH 10.0) + 20 mM deoxycholate + 15 mM β-CD (capillary 24.5 cm × 50 μm ID; 12 kV; 25°C)	UV-340 nm (OPA derivative)	[15, 16]

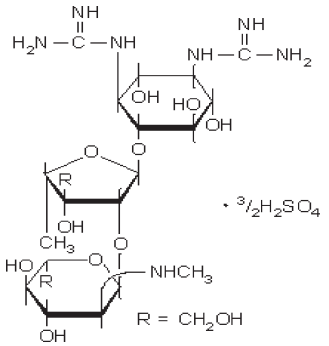


Table 1. Continued

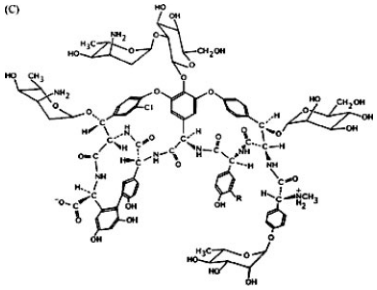
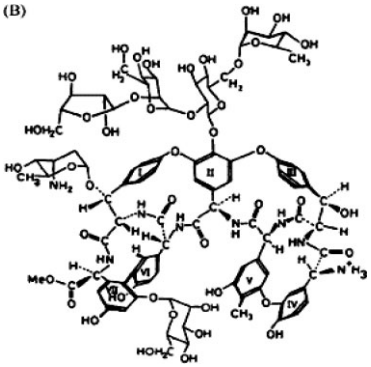
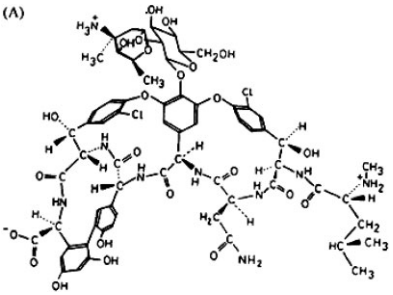
Group of antibiotic	Substance	Molecular formula (formula weight)[a)]	CAS number[b)]	Structure	CE separation conditions	Detection (LOD)	Reference
	Dihydrostreptomycin	$C_{21}H_{41}N_7O_{12} \cdot 3/2H_2SO_4$ (730.71)	5490-27-7	R = –CH_2OH	80 mM borate (pH 8.0) (capillary 60 cm × 75 μm ID; 15 kV; 35°C)	UV-185 nm (15 μg/mL)	[10]
	Streptomycin	$2C_{21}H_{39}N_7O_{12} \cdot (H_2SO_4)_3$ (1457.38)	3810-74-0	R = –CHO	20 mM phosphate-borate (capillary 47 cm × 100 μm ID; 25 kV; 25°C)	UV-200 nm (0.04 μg/mL)	[11]
Glycopeptide antibiotics	α-Avoparcin (R = –H) β-Avoparcin (R = –Cl)	–	–		20 mM borate (pH 9.2) + 75 mM SDS (capillary 50 cm (57 cm) × 75 μm ID; 15 kV; 30°C)	UV-200 nm (~0.01 μg/mL)	[19]
	Ristocetin A Ristocetin B	–	–	 Ristobiose[*O*-α-L-rhamnopyranosyl-(1–6)-*O*-β-D-glucopyranosyl] rather than ristotetrose[*O*-α-D-arabinofuranosyl-(1–2)-*O*-α-D-mannopyranosyl-(1–2)-*O*-α-L-rhamnopyranosyl-(1–6)-*O*-β-D-glucopyranosyl] is attached to ring II in Ristocetin B			
	Vancomycin	$C_{66}H_{75}Cl_2N_9O_{24}HCl \cdot xH_2O$ (1485.71)	123409-00-7				

Table 1. Continued

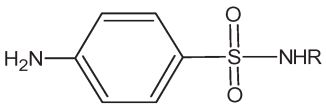
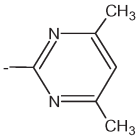
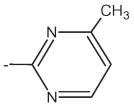
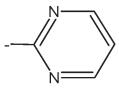
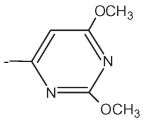
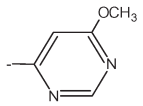
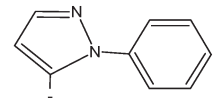
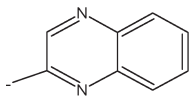
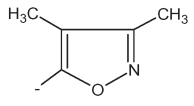
Group of antibiotic	Substance	Molecular formula (formula weight)[a)]	CAS number[b)]	Structure	CE separation conditions	Detection (LOD)	Reference
Sulfonamide antibiotics							
	Sulfamethazine	$C_{12}H_{14}N_4O_2S$ (278.33)	57-68-1	R = 	35 mM phosphate (pH 6.5) (capillary 72 cm (80.5 cm) × 50 μm ID; 25 kV; 25°C)	UV-205 nm (~0.005 μg/mL)	[20]
	Sulfamerazine	$C_{11}H_{12}N_4O_2S$ (264.30)	127-79-7	R = 			
	Sulfadiazine	$C_{10}H_{10}N_4O_2S$ (250.28)	68-35-9	R = 			
	Sulfadimethoxine	$C_{12}H_{14}N_4O_4S$ (310.33)	122-11-2	R = 			
	Sulfamonomethoxine	$C_{11}H_{12}N_4O_3S$ (280.30)	1220-83-3	R = 			
	Sulfaphenazole	$C_{15}H_{14}N_4O_2S$ (314.36)	526-08-9	R = 			
	Sulfaquinoaline	–	–	R = 			
	Sulfisoxazole	$C_{11}H_{13}N_3O_3S$ (267.30)	127-69-5	R = 			

Table 1. Continued

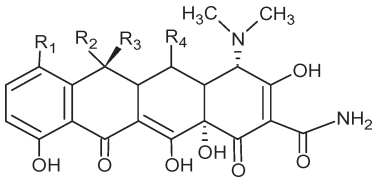
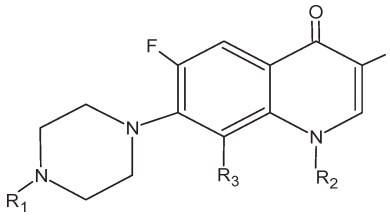
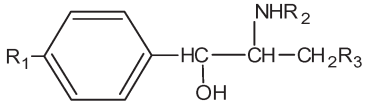
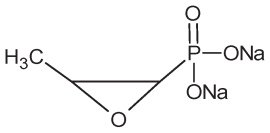
Group of antibiotic	Substance	Molecular formula (formula weight)[a)]	CAS number[b)]	Structure	CE separation conditions	Detection (LOD)	Reference
Tetracycline antibiotics							
	Tetracycline	$C_{22}H_{24}N_2O_8 \cdot xH_2O$ (444.43)	60-54-8	R_1 = –H, R_2 = $–CH_3$, R_3 = –OH, R_4 = –H	50 mM citric acid (pH 2.5) (capillary 37 cm × 75 μm ID; 10 kV; 25°C)	UV-260 nm (~0.002 μg/mL)	[21]
	Oxytetracycline	$C_{22}H_{24}N_2O_9 \cdot 2H_2O$ (496.46)	6153-64-6	R_1 = –H, R_2 = $–CH_3$, R_3 = –OH, R_4 = –OH			
	Doxycycline	$C_{22}H_{24}N_2O_8$ HCl 1/2 (H_2O) 1/2 (C_2H_6O) (512.94)	24390-14-5	R_1 = –H, R_2 = $–CH_3$, R_3 = –H, R_4 = –OH			
Fluoroquinolone antibiotics							
	Ofloxacin	$C_{18}H_{20}FN_3O_4$ (361.37)	82419-36-1	R_1 = $–CH_3$, R_2 = $–CH(CH_3)CH_2O–$ = R_3	50 mM phosphate (pH 2.8) + 40 mg/mL methyl β-CD (capillary 30 cm (37 cm) × 50 μm ID; 20 kV; 25°C)	UV-280 nm (0.003 μg/mL of each enantiomer)	[22]
	Enrofloxacin	$C_{19}H_{22}FN_3O_3$ (359.39)	93106-60-6	R_1 = $–CH_2CH_3$, R_2 =  -, R_3 = –H	25 mM phosphate-borate (capillary 77 cm × 75 μm ID; 25 kV; 25°C)	UV-280 nm (0.005–0.003 μg/mL)	[11]
	Ciprofloxacin	$C_{17}H_{18}FN_3O_3$ (331.34)	85721-33-1	R_1 = –H, R_2 =  -, R_3 = –H			
	Norfloxacin	$C_{16}H_{18}FN_3O_3$ (319.33)	70458-96-7	R_1 = –H, R_2 = $–CH_2CH_3$, R_3 = –H			
Other antibiotics							

Table 1. Continued

Group of antibiotic	Substance	Molecular formula (formula weight)[a)]	CAS number[b)]	Structure	CE separation conditions	Detection (LOD)	Reference
	Florphenicol	$C_{12}H_{14}Cl_2FNO_4S$ (358.21)	73231-34-2	$R_1 = –SO_2CH_3$, $R_2 = –COCHCl_2$, $R_3 = –F$	25 mM phosphate-borate (capillary 57 cm × 75 μm ID; 25 kV; 25°C)	UV-200 nm (0.015/0.008 μg/mL)	[11]
					50 mM borate (pH 9.0) + 25 mM SDS (capillary 52.5 cm (60 cm) × 75 μm ID; 15 kV; 20°C)	UV-214 nm	[23]
	Thiamphenicol	$C_{12}H_{15}Cl_2NO_5S$ (356.22)	15318-45-3	$R_1 = –SO_2CH_3$, $R_2 = –H$, $R_3 = –OH$			
	Chloramphenicol	$Cl_2CHCONHCH(CH_2OH)CH(OH)C_6H_4NO_2$ (323.13)	56-75-7	$R_1 = –NO_2$, $R_2 = –COCHCl_2$, $R_3 = –OH$	50 mM borate (pH 9.0) + 25 mM SDS (capillary 52.5 cm (60 cm) × 75 μm ID; 15 kV; 20°C)	UV-214 nm	[23]
	Fosfomycin	$C_3H_5O_4PNa_2$ (182.02)	26016-99-9		25 mM benzoic acid + 0.5 mM CTAB (pH 6.95 or 8.05) (capillary 56 cm (64.5 cm) × 50 μm ID; -25 kV; 25°C)	Indirect UV-254 nm (1 μg/mL)	[24]

Brij 35: lauryl polyoxyethylene ether.

a) Information obtained from (Sigma-Aldrich, http://www.sigmaaldrich.com; checked on May 2005).

b) CAS number: the number assigned by the Chemical Abstract Service (CAS) to identify specific chemical compounds. A chemical may have more than one CAS number. Not all chemicals have an assigned CAS number (California pesticide information portal terms and definitions, http://calpip.cdpr.ca.gov/cfdocs/calpip/prod/infodocs/glossary.cfm; checked on May 2005).

detection conditions employed and the LODs determined for the different antibiotics included in this review are indicated in the table. The results obtained in the analysis of antibiotics by CE are presented as follows, including first the most widely analyzed by this technique.

2.1 β-Lactam antibiotics

β-Lactam antibiotics can be classified into several groups according to their structural characteristics, but their unique structural feature is the presence of the four-membered β-lactam (2-azetidinone) ring. They include penicillins and cephalosporins (also aminocillins, carbapenems, and monobactams) (β-lactam antibiotics, http://www.cic.klte.hu/~gundat/betalaca.htm; checked on May 2005). Penicillins included in this review, ampicillin, penicillin V, penicillin G, amoxicillin, oxacillin, cloxacillin, dicloxacillin, nafcillin, and ticarcillin, have been separated using borate or phosphate-borate buffers at basic pH usually with SDS micelles [6–12]. As example, Fig. 1 shows the separation of a mixture of eight of these penicillins in borate buffer at pH 8 with SDS micelles in about 20 min after an on-column sample preconcentration by stacking [8]. In addition, the use of UV-detection at low wavelengths (from 185 to 214 nm) enabled to obtain LODs ranging from 0.005 to 1.5 μg/mL (see Table 1). The cephalosporins cephalexin and cefazolin have also been separated by CE. Cephalexin has been analyzed using borate buffer at pH 9.23 in combination with an anionic

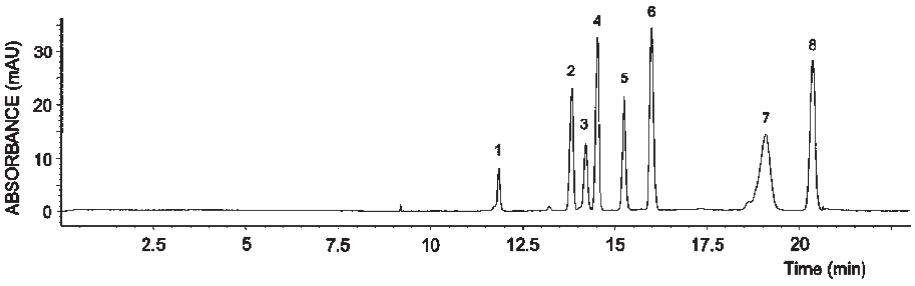


Figure 1. Separation of eight different penicillins by CE. Electrophoretic conditions: fused-silica capillary, l_d = 55.5 cm, l_t = 64 cm, and 75 μm ID; separation buffer, 20 mM borate (pH 8) containing 60 mM SDS; separation temperature, 25°C; applied voltage, 15 kV; hydrodynamic injection of 50 mbar for 360 s followed by reverse electrode polarity stacking (up to 95% of the buffer current intensity was reached). UV-detection at 210 nm. Peak identification: 1, amoxicillin; 2, penicillin G; 3, ampicillin; 4, oxacillin; 5, penicillin V; 6, cloxacillin; 7, nafcillin; 8, dicloxacillin. Reprinted from [8], with permission.

surfactant (SDS) and a neutral surfactant (lauryl polyoxyethylene ether, Brij 35) achieving LODs in the μg/mL range when UV-detection at 210 nm was used [13]. For the analysis of cefazolin, a phosphate buffer at pH 6.8 and UV-detection at 270 nm was employed [14].

2.2 Aminoglycoside antibiotics

Aminoglycoside antibiotics include a variety of related amino sugars joined *via* glycoside linkages. The aminoglycoside antibiotics included in this review are kanamycin, amikacin, tobramycin, gentamicin, sisomicin, netilmicin, as well as dihydrostreptomycin and streptomycin (see Table 1). Whereas most of these antibiotics are characterized by one main component accompanied by some minor components, gentamicin consists of four major components (C_1, C_{1a}, C_2, and C_{2a}). Because of the lack of UV chromophore groups in these antibiotics, derivatization is usually required prior to UV-detection. Thus, precolumn derivatization with 1,2-phthalic dicarboxaldehyde (OPA) was employed to analyze amikacin, tobramycin, gentamicin, sisomicin, and netilmicin using borate buffer at pH 10 in presence of the bile salt deoxycholate and the native β-CD used to enlarge the separation window [15, 16]. Under these experimental conditions, the separation of the four major components of gentamicin in a commercial sample from the derivatization agent (OPA) and an internal standard (IS) was achieved and it is illustrated in Fig. 2 as example. An interesting on-column derivatization with OPA was also performed to detect kanamycin B using borate buffer at pH 10 modified with methanol as BGE [17]. In addition, potential gradient detection was also employed for the analysis of gentamicin components when the separation was achieved with citrate buffer at pH 3.5 in presence of the cationic surfactant CTAB. This is a universal detector based on measuring the mobility differences between sample molecules and the separation buffer's co-ions, that is, the potential gradient along the axis of the capillary. LODs in the μg/mL range were achieved using this detection system [18]. The aminoglycosides dihydrostreptomycin and streptomycin were detected at the nonselective low wavelengths of 185 or 200 nm using borate or borate-phosphate buffers at basic pH (see Table 1). In fact, the analysis of these antibiotics using borate buffers at basic pH enables the formation of UV-absorbing borate complexes which can be detected by UV-detection. LODs of 15 and 0.04 μg/mL for dihydro-streptomycin and streptomycin, respectively, were reported [10, 11].

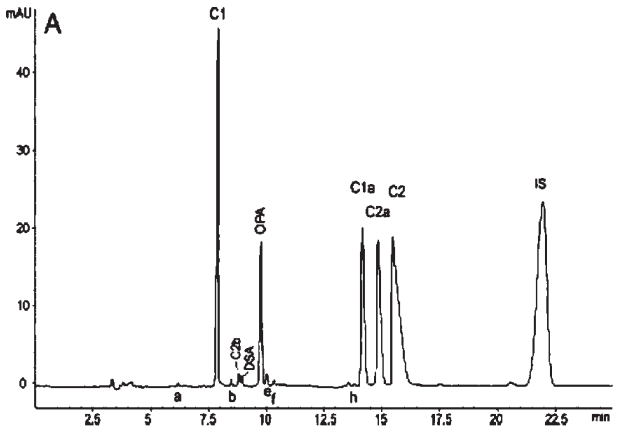


Figure 2. Separation of the major components of gentamicin by CE. Electrophoretic conditions: fused-silica capillary, l_d = 24.5 cm, l_t = 33.0 cm, and 50 μm ID; separation buffer, 100 mM borate (pH 10.0) containing 20 mM deoxycholate and 15 mM β-CD; separation temperature, 25°C; applied voltage, 12 kV; hydrodynamic injection 50 mbar for 5 s. UV-detection at 340 nm. Reprinted from [15], with permission.

2.3 Glycopeptide antibiotics

Glycopeptide antibiotics are composed of a peptide aglycone, neutral sugars, and an amino sugar. These aglycones consist of heptapeptides of cross-linked un-

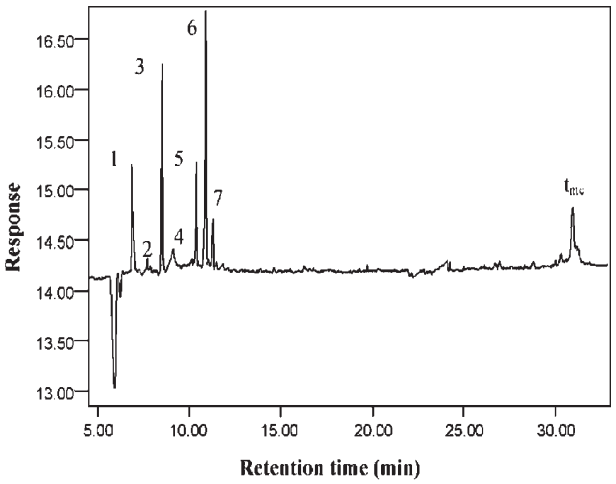


Figure 3. Separation of five glycopeptide antibiotics by CE. Electrophoretic conditions: fused-silica capillary, l_d = 50 cm, l_t = 57 cm, and 75 μm ID; separation buffer, 20 mM borate (pH 9.2) containing 75 mM SDS; separation temperature, 30°C; applied voltage, 15 kV; hydrodynamic injection for 15 s. UV-detection at 200 nm. Peak identification: 1, ristocetin A; 3, ristocetin B; 5, β-avoparcin; 6, vancomycin; 7, α-avoparcin; peaks 2 and 4 are minor components of ristocetin and avoparcin. Reprinted from [19], with permission.

usual aromatic amino acids, and conventional amino acids such as aspartic acid (see Table 1). Vancomycin, avoparcin, and ristocetin are members of this group of antibiotics. They are soluble in aqueous or polar organic solvents but insoluble in nonpolar organic solvents. Vancomycin, ristocetin, and avoparcin, the two latter in their two biological active forms (α- and β-avoparcin and ristocetin A and B), were separated by CE using borate buffer at pH 9.2 in presence of anionic micelles of SDS. Although these glycopeptides have similar p*I*s, they form borate complexes, which in the presence of the SDS micelles have sufficiently different net electrophoretic mobilities to allow their baseline separation as it is shown in Fig. 3. Due to their UV-absorption they were detected at 200 nm [19].

2.4 Sulfonamide antibiotics

Sulfonamide antibiotics are organic compounds possessing an amide of a sulfonic acid (see Table 1). The eight sulfonamides sulfamethazine, sulfamerazine, sulfadiazine, sulfadimethoxine, sulfamonomethoxine, sulfaphenazole, sulfaquinoaline, and sulfisoxazole were baseline separated with a 35 mM phosphate buffer at pH 6.5 in less than 15 min. They were detected registering their UV-absorption at 205 nm [20].

2.5 Tetracycline antibiotics

Tetracyclines are structurally related compounds with multiple functional groups with acid–base properties, whose presence confers them an amphoteric character. In fact, most of these compounds exhibit an p*I* between 4 and 6. Since in alkaline media tetracyclines oxidized easily, citric acid at pH 2.5 was used for the separation of the three tretracycline antibiotics tetracycline, oxytetracycline, and doxycyline obtaining good results in terms of resolution and sensitivity. The UV-absorption of these compounds at 260 nm was registered to detect them [21].

2.6 Fluoroquinolone antibiotics

Fluoroquinolone antibiotics form a group of broad-spectrum antibiotics derived from nalidixic acid. Enrofloxacin and norfloxacin, two of the most frequently used fluoroquinolones, were separated in about 11 min in a phosphate-borate buffer at basic pH [11]. In addition, the separation of the enantiomers of the chiral fluoroquinolone ofloxacin was achieved in phosphate buffer at acid pH (2.8) when methyl β-CD was used as chiral selector. Under these conditions, the detection of the enantiomers was performed at 280 nm [22]. This is the only enantiomeric separation of a chiral antibiotic by CE reported during the period of time reviewed in this article.

2.7 Other antibiotics

The antibiotics florphenicol, thiamphenicol, and chloramphenicol are structural analogs as it can be observed in Table 1. They were baseline separated in about 6 min using a borate buffer at pH 9 in presence of SDS micelles [23]. In addition, using phosphate-borate buffer the detection of florphenicol and thiamphenicol was performed at 200 nm [11].

Fosfomycin is an antibiotic possessing an epoxy moiety and phosphonic acid functions (see its structure in Table 1). This compound, which has very low molecular weight, two pK_as (2.0 and 6.4), and lack of absorption in the UV-region, was detected in the μg/mL range using indirect UV-absorption at 254 nm. Analysis of this antibiotic was achieved using benzoic acid as electrolytic solution containing a very low concentration of the cationic surfactant CTAB at neutral or slightly basic pHs depending on the sample analyzed [24].

3 Applications

The papers dealing with the analysis of antibiotics using CE as the separation technique during the period of time reviewed in this article were focused on the analysis of pharmaceutical preparations, biological samples, food samples, and environmental samples as it is illustrated in Table 2. This table shows the samples studied, the sample treatment followed, the antibiotics analyzed, and a brief description of the main conditions employed for their analysis by CE.

3.1 Antibiotics in pharmaceutical preparations

The analysis of antibiotics is of critical importance during their synthesis as well as for the quality control of their pharmaceutical preparations. Different pharmaceutical preparations were analyzed during the period of time reviewed in this work. They included formulations for preparing injection solutions, capsules, veterinary preparations, solutions, oral suspensions, as well as different drugs to assess their purity (see Table 2). Very easy sample treatments were followed to analyze these samples. Thus, aqueous or buffer solutions of β-lactam, aminoglycoside antibiotics, and thiamphenicol analogs, and solutions resulting from the derivatization of the aminoglycoside gentamicine were directly injected in the CE system.

Ampicillin was determined in powder for preparing injection solutions from two different manufacturers and in capsules using phosphate-borate buffer (pH 7.5) with SDS micelles after dissolution in phosphate buffer at pH 7.0. However, when preparing samples from capsules, solutions were sonicated and filtered prior to injection in the CE system. It was shown that this method was suitable for the routine control of purity of ampicillin bulk substances and pharmaceuticals, such as capsules and injections, with quantitation limits lower than 0.4% [6].

The simultaneous analysis of ticarcillin (β-lactam antibiotic/carboxypenicillin) and clavulanic acid (β-lactamase inhibitor), which are combined in Timentin preparations, was achieved using a phosphate-borate buffer (pH 8.7) with SDS micelles after dissolution of the pharmaceutical preparation in water. In this work, the quantitation limit of the antibiotic ticarcillin for the Timentin preparation was established in the concentration of 40 μg/mL [12].

The identification and simultaneous determination of dihydrostreptomycin and penicillin G (also its procaine salt) present in a multiantibiotic veterinary preparation (Veti-ps preparation) was achieved by CE using borate buffer at pH 8.0. In this work, sample solutions were prepared in water and diluted properly prior to the injection in the CE system achieving LOQs of 50 μg/mL for dihydrostreptomycin and 12 μg/mL for penicillin G [10].

The quantitative determination of chloramphenicol, florfenicol, and thiamphenicol in capsules and solutions was performed by CE using borate buffer (pH 9.0) containing SDS micelles. Water solutions were filtered prior to injection in the CE system. The analysis of different placebo mixtures showed that several excipients (lactose, talc, magnesium stearate, dextran, methyl parahydroxybenzoate *N*-methyl-2-pyrrolidone, propylene glycol, macrogol 300, and hypromellose) did not adversely affect the results [23].

The analysis of cephalexin in a commercially available oral suspension prepared by its appropriate dilution in water was achieved by CE using borate buffer at pH 9.2 with SDS and Brij 35 micelles. The sensitivity obtained (LOD ~ 2.4 μg/mL) in addition to the other analytical characteristics of the method were good enough to propose this methodology as an alternative to the official methods of analysis of cephalexin based on microbiologic assay and LC. The specificity of the electrophoretic method was demonstrated analyzing two samples of cephalexin (a simulated and a commercial sample) and the corresponding placebo observing that excipients of the suspension did not interfere with the cephalexin peak [13]. Figure 4 shows the electropherograms corresponding to a placebo from a simulated cephalexin oral suspension sample (Fig. 4A), a commercial sample (Fig. 4B), and a simulated sample (Fig. 4C).

The aminoglycoside antibiotic gentamicin was analyzed in 46 bulk samples of different manufacturers or pharmaceutical companies finding different patterns of the main components of gentamicin (C_1, C_{1a}, C_{2a}, and C_2) as well as many minor products associated to the existence of sisomicin. Almost all samples analyzed met the requirements established by the European and United States Pharmacopeias. The separation of these compounds was achieved in borate buffer at pH 10 containing deoxycholate and β-CD. The detection of gentamicin required a prederivatization step. Then, samples were derivatized with OPA in presence of methanol. These solutions after being vortexed and heated in a water bath at 40°C for exactly 4 min were diluted with methanol and cooled to room temperature prior to the injection in the CE system [16].

3.2 Antibiotics in biological samples

Biological samples usually contain interferences and proteinaceous components and particulate matter that make their analysis difficult. Therefore, a sample treat-

Table 2. Analysis of antibiotics by CE in different matrices

Application	Sample studied	Sample treatment	Analyte	CE conditions	Reference
Pharmaceutical preparations	Formulations in powder for preparing injection solutions and capsules	Direct injection of 20 mM phosphate buffer (pH 7.0) solutions (ultrasonication and filtration of capsules)	Ampicillin	Phosphate-borate (pH 7.5) + SDS (UV-200 nm)	[6]
	Injection preparation (Timentin)	Direct injection of water solutions	Ticarcillin and clavulanic acid	Phosphate-borate (pH 8.66) + SDS (UV-214 nm)	[12]
	Veterinary drugs	Direct injection of water solutions	Penicillin G, dihydrostreptomycin	Borate (pH 8.0) (UV-185 nm)	[10]
	Capsules and solutions	Direct injection of filtered water samples	Florphenicol, chloramphenicol, thiamphenicol	Borate (pH 9.0) + SDS (UV-214 nm)	[23]
	Oral suspensions	Direct injection of water samples	Cephalexin	Borate (pH 9.23) + SDS + Brij 35 (UV-210 nm)	[13]
	Drugs lots for purity assessment	Direct injection of derivatized solution	Gentamicin (OPA derivatives)	Borate (pH 10.0) + deoxycholate + β-CD (UV-340 nm)	[15, 16]
Biological samples	*In vitro* studies using the Caco-2 cell monolayers model	Direct injection of diluted samples obtained after LLE	Ofloxacin enantiomers	Phosphate (pH 2.8) + methyl β-CD (UV-280 nm)	[22]
	Plasma and microdialysis samples	Direct injection (microdialysis samples). Treatment with methanol and centrifugation prior injection (plasma samples)	Fosfomycin	Benzoic acid + CTAB (pH 6.95 for plasma and pH 8.05 for microdialysis samples) (indirect UV-254 nm and contactless conductivity detection)	[24]
	Serum, wound drains, and cerebrospinal fluids	Direct injection of filtered samples (centrifuged and frosted, being defrosted just before preparation)	Cefazolin	Phosphate (pH 6.8) (UV-270 nm)	[14]
	Human serum	Direct injection of water: methanol (1:1) solutions obtained after SPE	Gentamicin (C_1, C_{1a}, C_{2a}, C_2)	CHES (pH 9.5) + methanol (UV-230 nm)	[25]
	Tissue samples from poultry and porcine	Protein precipitation by ACN without or with LLE	Amoxicillin, doxycycline, streptomycin, thiamphenicol, florphenicol, enrofloxacin, ciprofloxacin, and norfloxacin	Phosphate-borate (UV-200–280 nm)	[11]
Food samples	Pork, chicken, and beef meat samples	Direct injection of ACN–water (50:50 v/v) solution after SLE + SPE	Sulfamethazine, sulfamerazine, sulfadiazine, sulfadimethoxine, sulfamonomethoxine, sulfaphenazole, sulfaquinoaline, sulfisoxazole	Phosphate (pH 6.5) (UV-205 nm)	[20]
Environmental samples	Farm water samples	Direct injection of filtered water samples	Amoxicillin, ampicillin, penicillin G, oxacillin, penicillin V, cloxacillin	Borate (pH 8.5) + SDS (UV-210 nm)	[7]
	Groundwater and surface water samples	Flow manifold coupled online to CE	Tetracycline, oxytetracycline, doxycycline	Citric acid (pH 2.5) (UV-260 nm)	[21]

LLE, liquid–liquid extraction; SLE, solid–liquid extraction.

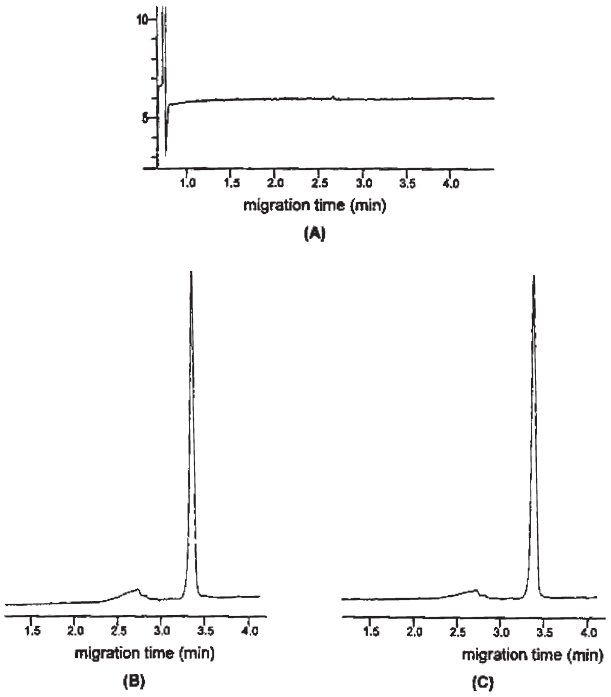


Figure 4. Analysis of samples of cephalexin oral suspensions by CE: (A) placebo from simulated sample; (B) commercial sample (oral suspension), 80 μg/mL; (C) simulated sample (oral suspension), 80 μg/mL. Electrophoretic conditions: fused-silica capillary, l_d = 28 cm, l_t = 50 cm, and 75 μm ID; separation buffer, 20 mM borate (pH 9.23) containing 20 mM SDS and 20 mM Brij 35; separation temperature, 30°C; applied voltage, 15 kV; sample injection 12.7 cm for 5 s. UV-detection at 210 nm. Reprinted from [13], with permission.

ment prior to the injection in the CE system is usually required [1]. Table 2 shows that biological samples reported in this review were treated by microdialysis, liquid–liquid extraction (LLE), solid–liquid extraction (SLE), SPE, centrifugation, and filtration before the injection in the CE system. In addition, biological fluids such as plasma and serum are generally easier to handle than solid samples such as tissue samples.

The quantitation of ofloxacin, which is a chiral fluoroquinolone for which the antibacterial activity of *S*-(−)-enantiomer is reported to be 8–128 times higher than that of the *R*-(+)-form, was achieved in physiological solutions using phosphate buffer at pH 2.8 and methyl β-CD as chiral selector. An LLE of ofloxacin with dichloromethane from physiological solutions (in Hank's balanced salt solution at pH 7.4) was performed to investigate the absorption of this antibiotic in *in vitro* studies (using Caco-2 cells as absorption model). The quantitation limits determined for the enantiomers were 11.4 ng/mL for *S*-ofloxacin and 10.8 ng/mL for the *R*-enantiomer [22].

The analysis of fosfomycin in human plasma and microdialysis samples collected from test persons during a clinical trial was carried out under reversed EOF conditions using benzoic acid and CTAB as BGE adjusted to pH 6.95 for plasma and to pH 8.05 for microdialysis samples. Although no sample preparation was needed for microdialysis samples, for plasma samples, proteins were precipitated with methanol and after centrifugation the supernantant was injected in the CE system. LODs ranged from 0.6 to 2 μg/mL, depending on the matrix and the detection method (indirect UV-detection at 254 nm and contactless conductivity detection) [24].

The cephalosporin cefazolin was determined in serum, contents of wound drains, and cerebrospinal fluid in a 24-h postoperative period after the administration of 1 g of the antibiotic just prior to skin incision in patients undergoing lumbar discectomy or craniectomy. The biofluid samples analyzed were centrifuged and frosted (at −18°C) until their analysis by CE. After defrosting, filtration through 0.45 μm syringe filters was made prior to the injection in the CE system. Analysis was performed using phosphate buffer at pH 6.8 and UV-detection at 270 nm [14].

The determination of gentamicin components (C_1, C_{1a}, C_{2a}, and C_2) in human serum was performed after an SPE of the sample. A water dilution of the serum sample was applied to the SPE cartridge (a weak cation exchanger) and then washed with 20 mM phosphate buffer at pH 7.4, 200 mM borate buffer at pH 9.0, and water, prior to the elution of gentamicin with ammonia/methanol. Then, after drying the eluate, it was reconstituted in water–methanol (1:1) and derivatized with 1,2-phthalic dicarboxaldehyde/mercaptoacetic acid prior to separation with CHES buffer at pH 9.5 containing 31.6% methanol and UV-detection at 230 nm. Under these conditions SPE recoveries ranging from 78 to 93% were obtained and LODs about 0.3 μg/mL for the four gentamicin components were achieved. Therefore, sufficient sensitivity for the total gentamicin was achieved by this CE method since the permitted maximum trough and peak concentrations of gentamicin in clinical practice are 2 and 10 mg/L, respectively. Figure 5 depicts the electropherograms obtained by the SPE-CE method for human serum samples of patients with concentration of gentamicin of 1.47 mg/L (below the toxic concentration) and 12.3 mg/L (above the toxic concentration) [25].

The quantitative determination of antibiotic residues in poultry and porcine tissues for eight of the most frequently used antibiotics and the drug nifursol (see Table 2) was achieved by CE after a simple extraction with ACN or ethyl acetate under basic conditions. Thus, the samples including enrofloxacin, ciprofloxacin, norfloxacin, and nifursol

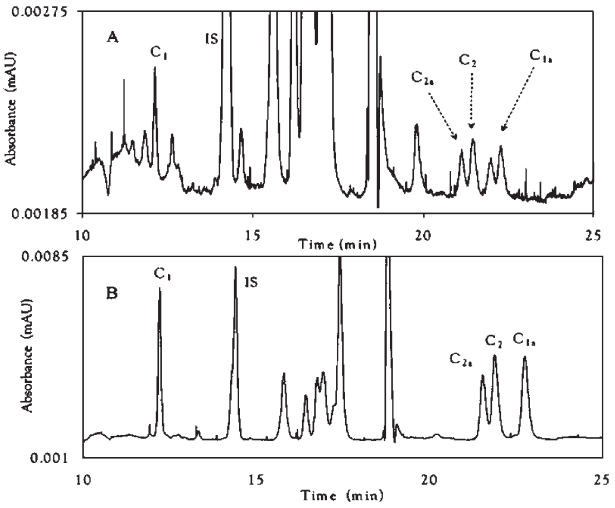


Figure 5. Analysis by SPE-CE of a human serum with concentrations of gentamicin of 1.47 mg/L (A) and 12.3 mg/L (B), both containing 5 mg/L of furosemide as IS. Electrophoretic conditions: fused-silica capillary, l_d = 40 cm, l_t = 50.2 cm, and 75 μm ID; separation buffer, 60 mM CHES (pH 9.5) containing 31.6% m/v methanol; separation temperature, 20°C; applied voltage, 23 kV; sample injection, 0.8 psi for 10 s. UV-detection at 230 nm. Reprinted from [25], with permission.

were only deproteinized by precipitacion of the proteins using ACN, while the samples containing amoxicillin, doxycycline, streptomycin, thiamphenicol, and florphenicol were deproteinized by ACN and subsequently extracted with ethyl acetate in the presence of 1 M NaOH. Then, the organic solvent was evaporated to dryness and the dry residue was reconstituted in 2 mM borate (0.5 mL), centrifuged, and injected into the CE system. The analysis with phosphate-borate buffer and UV-detection from 200 to 280 nm depending on the antibiotic enabled to achieve LODs from 3 to 35 ng/mL [11].

3.3 Antibiotics in food samples

Antibiotics orally administered or mixed with animal feed have been used to prevent and control a number of diseases in veterinary practice. The use of these substances as well as the maximum residue levels in animal foodstuffs are regulated because of the concerns about their possible effects on human health [26]. As a consequence, the determination of the residue levels in meat and other animal byproducts (*i.e.*, milk and eggs) used for human consumption is an important task.

The determination of eight commonly used sulfonamides (see Table 2) in meat samples was performed by CE using phosphate buffer at pH 6.5. A solvent extraction with ACN followed by an SPE procedure for sample cleanup and preconcentration of sulfonamides was employed prior to CE analysis. Figure 6 illustrates the electropherograms corresponding to a sulfonamide (100 μg/kg) spiked beef sample and a pork sample where the sulfonamine antibiotic sulfisoxazole in presence of an IS (1-naphthoxyacetic acid) was detected. In addition, the detection limits (from 5 to 10 μg/mL) and quantitation limits for this method are low enough to determine residues of these drugs in meat samples below the allowed maximum residue limits established by the European Community [20].

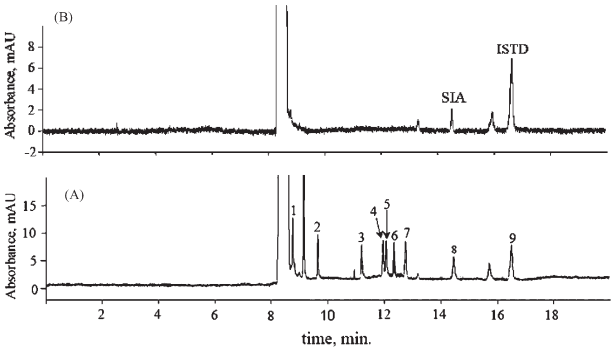


Figure 6. Analysis by CE of a sulfonamide (100 μg/kg) spiked beef sample (A) and a pork sample (containing sulfisoxazole (SIA) and 1-naphthoxyacetic acid (ISTD)) (B). Electrophoretic conditions: fused-silica capillary, l_d = 72 cm, l_t = 80.5 cm, and 50 μm ID; separation buffer, 35 mM phosphate (pH 6.5); separation temperature, 25°C; applied voltage, 25 kV; sample injection, 50 mbar for 15 s. UV-detection at 205 nm. Peak identification: 1, sulfamethazine, 2, sulfamerazine, 3, sulfadiazine, 4, sulfadimethoxine, 5, sulfamonomethoxine, 6, sulfaphenazole, 7, sulfaquinoaline, 8, sulfisoxazole, 9, 1-naphthoxyacetic acid. Reprinted from [20], with permission.

3.4 Antibiotics in environmental samples: their role as emerging contaminants

The determination of antibiotic residues in environmental samples is interesting because they are suspected of being responsible for the appearance of bacterial strains that are resistant to antibiotics [7]. In addition, it is important to consider that the large amount of antibiotics which are continuously introduced to the environment by numerous routes make them potential pollutants, called as emerging pollutants, included in the denominated pharmaceutical and active ingredients in personal care products (PPCPs). Regardless of how short their half-lives in the environment might be, however, all PPCPs can act as "persistent" pollutants because they are replenished by the continuous introduction of sewage effluents (http://

www.epa.gov/nerlesd1/chemistry/ppcp/images/iom-2003.pdf; checked on May 2005). The most prevalent antibiotics found in the environment have been macrolide, fluoroquinolone, and sulfonamide groups [27]. Although tetracyclines or penicillins have been found only in some cases and generally at low concentrations, the two applications found in the literature in the period of time reviewed in this work are focused on the determination of these antibiotics in water samples.

The separation and determination of penicillins in farm water samples were performed by CE using borate buffer at pH 8.5 with SDS micelles and UV-detection at 210 nm. In this study, a very simply sample treatment was performed since the water samples were only filtered before introduction in the CE equipment. Under these conditions LODs of about 0.2 μg/mL were achieved although, as the authors indicated, they would be improved if a solid-phase concentration step is included into the sample preparation process [7].

Three tetracyclines (tetracycline, oxytetracycline, and doxycycline) were determined in groundwater and surface water samples after their preconcentration on a STRATA-X solid-phase minicolumn inserted in a flow manifold coupled online to CE. Figure 7 shows the electropherogram corresponding to a water sample spiked with a 5 ng/mL concentration of each tetracycline derivative. This CE method enabled the detection up to 2 ng/mL of tetracyclines in water samples [21].

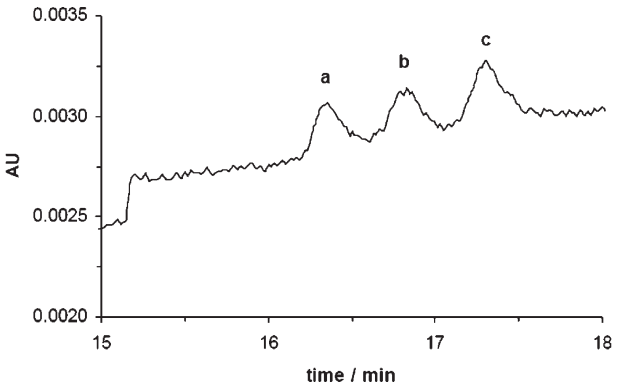


Figure 7. Analysis by CE of a water sample spiked with 5 ng/mL of each analyte. Electrophoretic conditions: fused-silica capillary, l_d = 37 cm and 75 μm ID; separation buffer, 50 mM citric acid (pH 2.5); separation temperature, 25°C; applied voltage, 10 kV; sample injection 20 psi for 10 s. UV-detection at 260 nm. Peak identification: a, tetracycline; b, oxytetracycline; c, doxycycline. Reprinted from [21], with permission.

4 Conclusions and future prospects

The separation of mixtures of antibiotics has been successfully performed by CE, mainly using MEKC. The lack of sensitivity for some classes of antibiotics, such as aminoglycoside antibiotics, has been overcome derivatizing them to form UV-absorbing derivatives or UV-absorbing borate complexes or using alternative detection systems. Although detection by direct UV-absorption of antibiotics or their derivatives was used in most of the papers included in this review, the use of alternative detection systems such as MS or electrochemical detection to solve sensitivity problems for the analysis of some antibiotics constitute a future prospect in this field.

Although the sample preparation required for the determination of antibiotics in pharmaceutical preparations has usually been simple, a more elaborated sample treatment before the injection in the CE system is usually needed for the analysis of antibiotics in complex matrices, *i.e.*, in biological, food, and environmental samples. In this sense, offline sample treatment by SPE, LLE, and SLE has been performed. However, the online coupling of sample treatment systems to CE seems to be very promising because it enables the automatization of the analytical process, one interesting example being included in this review [21]. In addition, these sample treatment procedures can concentrate antibiotic samples prior to the injection in the CE system. It is also important to remark the interest of the use of on-column sample preconcentration techniques based on electrophoretic principles, such as stacking preconcentration, which has been achieved very recently for a group of penicillins [8]. In fact, the achievement of low detection limits is important for the analysis of biological, food, and environmental samples. Thus, the maximum residue levels of antibiotics in foods are in the μg/mL level [26] and the concentrations of antibiotics in environmental samples may range from the μg/mL to the pg/mL levels.

The development of new applications for antibiotics, especially in the environmental field where their determination at low concentration levels has an increasing interest, is also a future prospect in the analysis of antibiotics by CE.

Finally, another trend in analytical chemistry that also affects the analysis of antibiotics by CE is the transfer of CE methods to miniaturized systems such as microchips. As an illustrative example, Fig. 8 shows the separation of two penicillin antibiotics in seconds when their detection at the micromolar concentration was performed by amperometric detection [28].

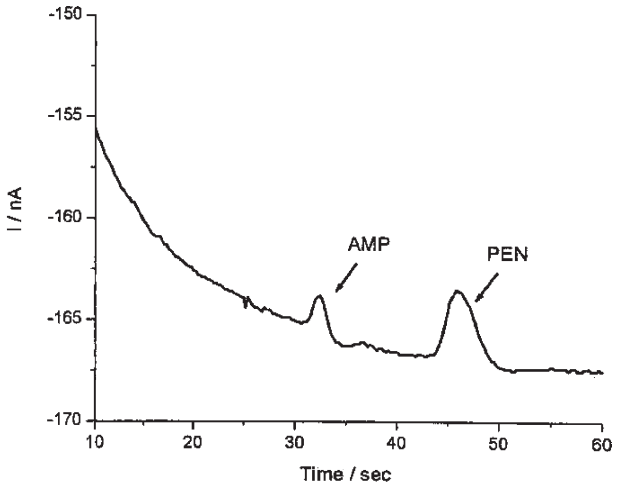


Figure 8. Separation of 13 μM ampicillin (AMP) and 12 μM penicillin G (PEN) by CE and pulsed amperometric detection. CE chip with channels of 50 μm width, 50 μm deep and sample loop 580 mm long. Separation buffer, 10 mM borate (pH 9.45); applied voltage, 1.7 kV; sample injection for 10 s. Pulsed amperometric detection using a potential of 0.5 V. Reprinted from [28], with permission.

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