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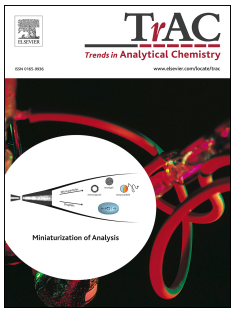

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Determination of aminoglycoside antibiotics: current status and future trends

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Abstract

The use of aminoglycoside antibiotics is prevalent in medicine and agriculture. Their overuse increases their mobility in the environment, resulting in a need for reliable methods for their determination in a variety of matrices. However, the properties of aminoglycosides, in particular their high polarity, make the development of such methods a non-trivial task, inciting researchers to tackle this complex issue from different angles. The necessity to determine aminoglycosides in complex matrices and at low concentration levels requires the development of relatively elaborate sample preparation methods and the use of selective and sensitive detection techniques. Various modes of liquid chromatography coupled with tandem mass spectrometry are usually the analytical methods of choice. However, the recent developments in techniques such as bioassays, quantum dot-based colourimetric applications and various aptasensors point towards the development of more easily accessible and user-friendly point-of-need tests for screening applications in food control and environmental monitoring. This review summarizes the state-of-the-art in sample preparation protocols and the determination of aminoglycosides using various techniques and outlines the future trends with an emphasis placed on the novel and emerging solutions in this area.

Keywords: aminoglycoside antibiotics, liquid chromatography, novel trends in sample preparation, residue analysis.

Abbreviations

2D-LC	two-dimensional chromatography
ABS	Acid Chrome Black Special
AD	amperometric detector
AGs	aminoglycoside antibiotics
AMI	amikacin
APR	apramycin

31	AQC	6-aminoquinolyl-N-hydroxysuccinimidyl carbamate
32	AVI	avilamycin
33	BAC	bacitracin
34	BEK	bekanamycin
35	CAD	charged aerosol detection
36	CBX	carboxylic acid sorbent
37	CE	capillary electrophoresis
38	CFSE	6-carboxyfluorescein succinidyl ester
39	C^4D	contactless conductivity detection
40	DC-ELISA	direct competitive enzyme linked immunosorbent assay
41	DHSTR	dihydrostreptomycin
42	DMIP	dummy molecularly imprinted polymer
43	DSPE	dispersive solid phase extraction
44	ELISA	enzyme linked immunosorbent assay
45	ELSD	evaporative light scattering detection
46	FASS	field-amplified sample stacking
47	FDNB	1-fluoro-2,4-dinitrobenzene
48	FESI	field-enhanced sample injection
49	FIA	fluoroimmunoassay
50	FLD	fluorescence detector
51	FMOC	9-fluorenylmethyloxycarbonyl
52	GCB	graphitized carbon black
53	GEN	gentamycin
54	HFBA	heptafluorobutyric acid
55	HILIC	hydrophilic interaction chromatography
56	HLB	hydrophilic-lipophilic balance
57	HPLC	high performance liquid chromatography
58	HYG	hygromycin
59	IPLC	ion-pairing liquid chromatography
60	IC-ELISA	indirect competitive enzyme linked immunosorbent assay
61	ISE	ion-selective electrode
62	KAN	kanamycin
63	LIF	laser-induced fluorescence detection
64	LIN	lincomycin

65	LIV	lividomycin
66	LLE	liquid-liquid extraction
67	MCX	medium cationic exchangers
68	MIP	molecularly imprinted polymers
69	MS	mass spectrometry
70	MSB	moving substitution boundary
71	MS/MS	tandem mass spectrometry
72	NEO	neomycin
73	NET	netilmicin
74	NITC	1-naphthyl isothiocyanate
75	NMR	nuclear magnetic resonance spectroscopy
76	NP	nanoparticle
77	OPA	o-phthalaldehyde
78	OPD	o-phenylenediamine
79	PAD	pulsed amperometric detection
80	PAR	paromomycin
81	PBS	phosphate-buffered saline
82	PCX	polymeric cation exchanger
83	PFPA	pentafluoropropionic acid
84	PSA	primary-secondary amine
85	PTFE	polytetrafluoroethylene
86	QD	quantum dots
87	RIA	radioimmunoassay
88	RIB	ribostamycin
89	SCX	strong cation exchange
90	SIS	sismocin
91	SPC	spectinomycin
92	SPE	solid phase extraction
93	STR	streptomycin
94	TFA	trifluoroacetic acid
95	TMB	3,39,5,59-tetramethylbenzidine
96	TOB	tobramycin
97	VAL	validamycin
98	WCX	weak cationic exchangers

ZIC-HILIC stationary phases with zwitterionic groups covalently bound to the surface of silica particles

1. Introduction

Aminoglycosides antibiotics (AGs) are a group of pharmaceuticals with a broad spectrum of therapeutic applications [1]. AGs are mainly used against infections caused by Gram-negative and less often Gram-positive bacteria. The first AG was discovered by A. Schatz and S. Waksman in 1943. They, for the first time, isolated streptomycin from *Streptomyces griseus* microorganisms and noted its antimicrobial properties. Their discovery was honoured with the Nobel Prize in 1952. With regard to their origin, AGs are classified into two groups: natural (e.g. neomycin, gentamycin, kanamycin, streptomycin, tobramycin, sisomycin) and semi-synthetic (e.g. amikacin, dibekacin, isepamycin, netilmycin, arbekacin) antibiotics.

Aminoglycoside antibiotics are a group of drugs with very uniform pharmacokinetic properties. They are used both in human therapy and in veterinary treatment. The spectrum of therapeutic usages of AGs includes infections of the urinary system, respiratory tract and also bones, joints and skin infections. AGs are also used in ophthalmology as well as for sterilization of the gastrointestinal tract prior to surgery. Moreover, they are used to supplement feed intended for farm animals and in gardening for pest control [2,3]. Due to the polycationic character of their molecules, AGs are characterized by poor absorption after oral administration. Therefore, AGs are administered intramuscularly (injections), through the skin (creams) and directly into the eyes and ears (drops). The mechanism of AGs action consists of their bonding with A-site of bacterial ribosome (or protein ribosome) and disrupting protein translation which ultimately leads to bacteria's cell death [1].

Despite their high antimicrobial efficiency, aminoglycosides are also classified as toxic substances with low therapeutic indices. For example, the ratio of tobramycin concentration in plasma causing toxic effects to the therapeutic range is approximately 50% [4]. Furthermore, AGs are able to accumulate in parenchymal tissues e.g. in the renal cortex (by bonding to glycoproteins, like megalin), which negatively affects the urinary tract function. Side effects of AGs include oto- and nephrotoxicity, and damage to the digestive and nervous system. Additionally, AGs may cause fetal damage due to their ability to pass through the placenta. Therefore, the kind of AG and its dosage must be strictly controlled.

Due to their low cost, there is a danger of the overuse of these drugs in commercial animal farms. AGs are used not only for the animals' treatment but also as preventive measures and as growth promotors, especially in large-scale farms, with the use of AGs in veterinary applications amounting to approx. 3.5% of the overall antibiotics use [5]. If not

managed properly, this can have an adverse impact on the environment, since AGs are non-metabolizable agents and are thus excreted as unchanged molecules [6]. The release of large amounts of AGs into the environment leads to the increase of antibiotic resistance in some strains of bacteria (especially enteric bacteria) [7]. Half-lives of AGs range from 2 to 3 hours in plasma and from 30 to 700 hours in tissues [8]. Since they accumulate in animal tissues, AGs may be found in food of animal origin. The awareness of this issue has been raised following the publicised issue of agricultural antibiotics overuse in North Carolina (USA), where large-scale breeding farms are located, which led to significant amounts of antibiotics being found in wastewater from farms, soil and animal tissues. In response, many countries have introduced legislation which sets limits on the maximum residue content of antibiotics in foods of animal origin (see Table 1). Additionally, the largest restaurant franchises such as McDonald's, KFC and Subway are now claiming that the food that they offer does not contain antibiotics.

Table 1. Maximum residue limits (MRL) of selected aminoglycoside antibiotics in food of animal origin set by the EU regulations [9].

Aminoglycoside	**Food origin (animal species)**	**MRL [µg/kg]**
APR	Bovine	20000 (kidney) 10000 (liver) 1000 (fat, muscle)
STR	Ruminants Rabbit Porcine	1000 (kidney) 500 (fat, liver, muscle) 200 (ruminants milk)
DHSTR	Ruminants Rabbit Porcine	1000 (kidney) 500 (fat, liver, muscle) 200 (ruminants milk)
NEO	All species	50000 (kidney) 500 (fat, liver, muscle, eggs) 1500 (milk)
GEN (sum of C1, C1a, C2 and C2a)	Bovine Porcine	750 (kidney) 50 (muscle, fat) 200 (liver)
AVI	Porcine	200 (kidney)

	Poultry	100 (fat)
	Rabbit	50 (muscle)
		300 (liver)
KAN	All species (except finfish)	2500 (kidney)
		100 (muscle)
		600 (liver)
		150 (milk)

The excretion of AGs in the unchanged forms and inflow of AGs-containing wastewaters from the pharmaceutical industry and hospitals facilitates their mobility in the environment and increases the levels of surface waters and soil pollution. Appropriate handling of this type of pollutants is a critical problem and requires special both dedicated legislation and specialised treatment facilities. Schematic illustration of AGs' mobility in the environment is shown in Fig. 1.

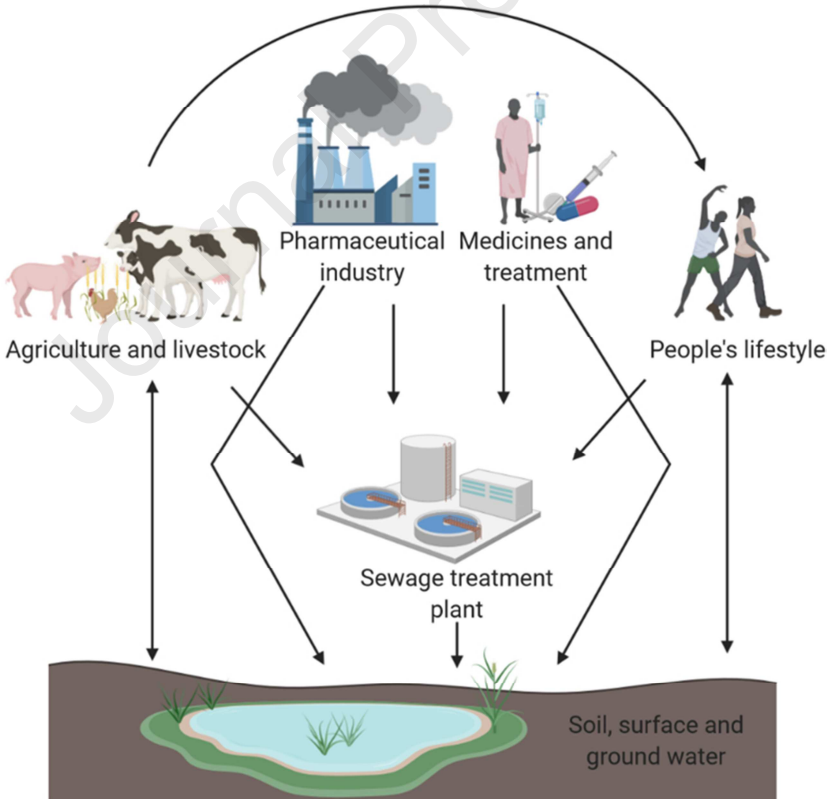

Fig. 1. Mobility of aminoglycoside antibiotics in the environment. Created with BioRender.com.

AGs are weak bases consisting of two or more molecules of aminosugars (D-glucosamine, D-kanosamine) connected by glycosidic bond with cyclitol in the form of (i) streptidine (e.g

streptomycin), (ii) 4,5-di-substituted deoxystreptamine (e.g. neomycin) or (iii) 4,6-di-substituted deoxystreptamine (e.g. kanamycin) (see Fig. 2). They are characterized by high polarity and hydrophilicity (logP values in the range from -4 to -9), very soluble in water, slightly soluble in methanol and insoluble in non-polar organic solvents. Some of AGs occur in the form of complexes, composed of several different chemical compounds. For example, gentamycin consists of 4 main compounds, such as gentamycin C1 (477.6 g/mol), gentamycin C1A (449.5 g/mol) and gentamycin C2 in the form of two stereoisomers A and B (463 g/mol). Another example is neomycin, which consists of two stereoisomers B and C, where only neomycin B has found therapeutical usage [8]. Due to their high polarity, polycationic character and lack of chromophores, the analysis of aminoglycosides is a challenging task both at the sample preparation and final determination stages.

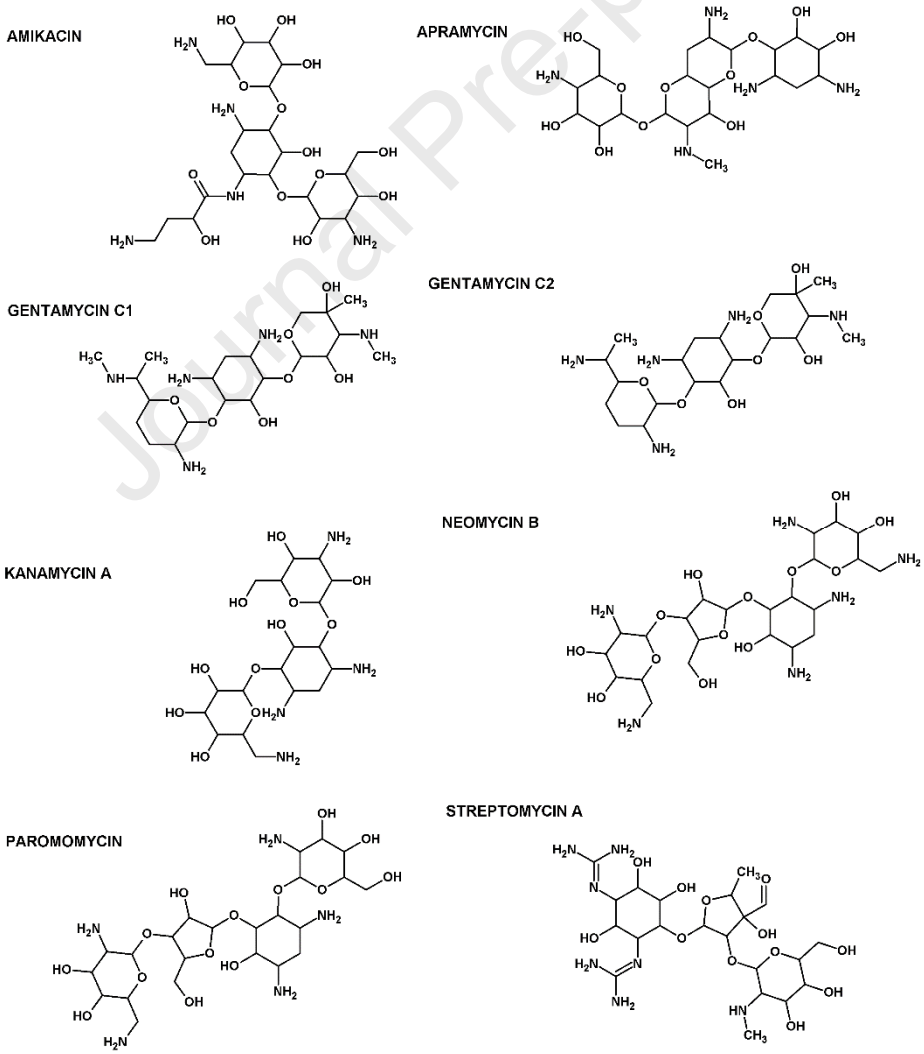

Fig. 2. Structures of selected aminoglycoside antibiotics.

2. Determination of aminoglycosides using liquid chromatography coupled to various detectors

Liquid chromatography (LC) remains the gold standard in the separation of AGs prior to their determination. This is not only due to the fact that it is a well-established technique with plenty of literature to fall upon when confronted with the issue of analysing a particular type of samples but also due to the possibility to analyse several AGs at the same time – a trait common to perhaps only capillary electrophoresis in the context of the analysis of aminoglycosides in complex matrices. While the choice of the most suitable method necessarily depends on the analytical task at hand and the type of the sample, LC-based techniques seem to be the most commonly used due to their high resolution, selectivity and sensitivity.

2.1. Sample preparation and clean-up methods for LC analysis

The physicochemical properties of aminoglycosides and the complexity of the usual matrices is the source of numerous issues with their determination, leading to irreproducible and inaccurate results, and so sample preparation is a crucial step in the analytical process.

In the case of relatively simple matrices such as pharmaceutical formulations, the sample preparation protocol is usually limited to the sample dissolution with deionized water, or with the mobile phase used in further LC investigation. In particular, such procedures are used for e.g. eye drops, tablets (after grinding) and some types of medicated animal feed [10–13]. In the case of creams and ointments, additional de-fatting step is usually required. Various non-polar organic solvents (e.g. DCM) are used for this purpose [11]. When it comes to matrices such as foods of animal origin, the sample preparation procedures tend to be more complicated. For instance, honey, consisting mainly of sugars, may cause problems during sample clean-up and analysis due to the presence of enzymes as well as polyphenols [14]. In the case of milk or animal material foods, the substantial amounts of proteins, fats, salts, vitamins and minerals may also interfere with isolation and determination of AGs. The established and emerging sample preparation methods used for determination of AGs in a variety of matrices are summarized in Table 2, while a generalised sample preparation scheme is shown in Fig. 3. It should be noted, however, that the optimal approach to sample treatment for the determination of AGs necessarily depends on numerous factors such as the desired application, complexity of the sample matrix, concentration of analytes, etc. As such, there is

no one-approach-fits-all solution, and the particular considerations which should be made when selecting the sample treatment approach are outlined in the following sub-sections.

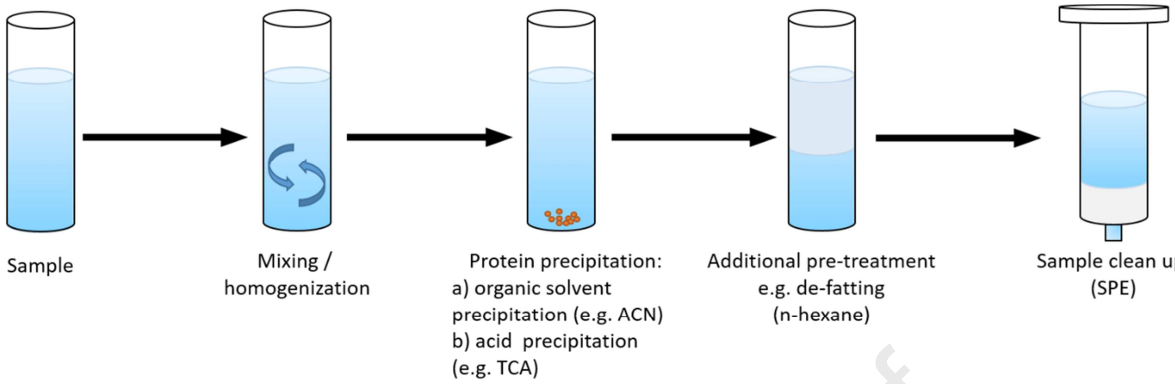

Fig. 3. Generalised scheme of sample treatment for subsequent determination of aminoglycosides.

Sample pre-treatment

The first step in the sample preparation protocol is usually sample mixing and homogenization or grinding. If large amounts of proteins are present, they are removed by precipitation, either with the use of organic solvents such as methanol, or acetonitrile [15–18]. It has to be noted, however, that AGs are poorly soluble in mixtures containing high amounts of organic solvents which can lead to their losses during protein precipitation. On the other hand, AGs show high stability and good solubility in acidic aqueous solutions. Acid precipitation seems therefore to be a safer alternative for protein removal in the context of AGs determination. Chlorinated or fluorinated organic acids such as trichloroacetic acid (TCA), trifluoroacetic acid (TFA) or heptafluorobutyric acid (HFBA) [18–27] are commonly used for this purpose due to their high efficiency at relatively low concentrations. It seems that TCA is the most frequently used precipitating agent owing to its low price and fast action. Typically, 2÷5% TCA solutions are used [28]. Precipitating solutions may contain other components, such as chelating agents (EDTA) to break down AGs complexes with polyvalent ions [22,24,27,29], pH control compounds (NH_4Ac, KH_2PO_4) [22,28–30]) or ionic strength fixating substances (NaCl) [22,26,29]). After protein precipitation, samples are centrifuged/filtered and defatted when necessary [23,26]. Finally, the pH of the sample solutions may be set to the desired value [25,28,29].

Sample purification and enrichment

The pre-treatment of the sample is followed by a purification and enrichment stage using a variety of techniques, chief among them SPE. Cationic exchangers (mostly weak cationic exchangers - WCX), C18 sorbents (in combination with ion-pairing reagents) or hydrophilic-lipophilic balance mode (HLB) SPE sorbents are commonly used. The selection of appropriate sorbent type can be challenging, particularly in the case of multiple analytes that need to be processed simultaneously, due to different values of acid/base dissociation constants for various AGs.

Ion exchange sorbents are frequently employed due to the poly-cationic character of AGs and their affinity to the functional groups (Table 2). This type of sorbents can be used for purification of many sample types including animal tissues (kidney, liver, muscle), milk, eggs, honey, royal jelly and animal feeds [14,24,29,31]. Extraction protocols with WCX cartridges require pH in the range of 6÷8. Under such conditions, functional groups of sorbent are negatively charged and attract protonated AGs molecules. At lower pH values (< 3), the sorbent surface becomes neutral, facilitating AGs elution. The ionic strength of the pre-extraction mixture may have a notable impact on the recoveries, at least in the case of some analytes [29]. Additional issues might occur when several AGs are to be extracted at the same time since in certain matrices the signal for particular AGs might be suppressed depending on the type of the cartridge used [31]. However, it was shown that polymeric cation exchangers with strong cation exchange functionality (PCX) provide better results in the case of select AGs compared to standard WCX cartridges [14], while in the case of the extraction of weakly basic compounds such as gentamycin, strong cation exchange (SCX) cartridges perform better altogether [24].

Similarly to ion exchange sorbents, hydrophilic-lipophilic balance (HLB)-based sorbents show AGs extraction efficiency varying with the pH of sample extracts (Table 2). This issue could be resolved e.g. through developing multi-step extraction protocols [21] or by synthesizing novel sorbents. The latter path was taken in a study in which urea-formaldehyde resin has been synthesized inside a small internal diameter PTFE tube [25]. The resulting monolithic microcolumn was used as a sorbent for determination of streptomycin, neomycin and tobramycin in fish meat extracts. In a different approach, four monolithic poly(methacrylic acid-co-ethylene methacrylate) fibers were bunched together and used for extraction of 6 AGs from honey and milk samples. Due to polymeric nature and carboxyl functionality present on the surface of the fibers, two types of sorption mechanisms were

observed: hydrophobic interactions and cation-exchange mechanism [19], and thus the extraction process was greatly simplified.

Sample clean-up can also be carried out using non-polar octadecyl (C18) cartridges (see Table 2). Since AGs, due to their hydrophilic nature, interact very weakly with hydrophobic C18 sorbents, the latter may be used to remove non-polar components of sample extracts. Such clean-up may be performed e.g. using dispersive SPE (DSPE) [3] and, since it is relatively fast, incorporated into QuEChERS protocols [32]. Conversely, the polycationic character of AGs makes it is possible to increase their hydrophobicity by the creation of ion pairs with reagents such as perfluorinated organic acids (e.g. heptafluorobutyric acid, HFBA). Such ion pairs may be isolated from extracts using techniques commonly used for other non-polar substances. In particular, both SPE and liquid chromatography based on reversed-phase principle can be used [33].

The latest trends in AGs extraction/sample clean-up take advantage of molecularly imprinted polymers (MIPs) and magnetically active sorbents (Table 2). MIPs are tailored, highly selective sorbents fabricated in the process of spatially constrained polymerization at the molecular level. The template (analyte) molecule interacts with the monomer functional groups (e.g. by ionic, hydrogen or covalent bonds) forming complexes. After cross-linking, the shape of such a complex is trapped/imprinted in the three-dimensional polymer structure. The template/analyte is then purged from the polymer leaving a molecular imprint. The resulting material shows high affinity to molecules shaped similarly to the template (analyte) molecules. In the case of MIPs, the analyte-sorbent interactions are relatively strong, therefore it is possible to use 2- or 3-step washing procedures (with MeOH or DCM) during sample preparation. Thorough washing helps reduce interferences caused by both polar and non-polar matrix components. Unfortunately, only one type of such sorbent is commercially available at the time of writing (SupelMIP® SPE-AG from Supelco). Sample clean-up procedures employing MIPs were used for AGs determination in animal tissues, fish, eggs, processed food, honey, milk and milk-based food products [27,34]. The MIP sorbents can be re-used dozens of times without the use of sorption efficiency [35], and enable overall better recoveries compared to conventional SPE.

Magnetically active sorbents consist of some sort of ferromagnetic particles (most commonly Fe_3O_4) covered with the layer of the actual sorptive material. The sorbents of that type are used for dispersive solid-phase extraction (DSPE). The small particle size of such

sorbents makes it easier to achieve higher extraction efficiencies mostly due to the high specific surface area. The magnetic activity of sorbent particles facilitates its separation from the sample matrix. Unfortunately, until now sorbents of this type are not commercially available and the literature concerning their applications is scarce. However, some promising applications include the development of a poly(vinyl alcohol)-coated core-shell magnetic nanoparticles (Fe_3O_4) for the DSPE extraction of three AGs from honey [36] and the use of a similar approach in which the Fe_3O_4 nanoparticles modified with carbohydrates with functional groups chosen to mimic AGs structure and properties [37]. The relatively high recovery values obtained in both scenarios (83% to 101% and 94% to 109%, respectively) highlight the potential of this approach to the extraction of AGs from various matrices.

304 Table 2. Sample preparation and clean-up procedures for determination of aminoglycoside antibiotics in a variety of matrices.

Type of SPE sorbents	Aminoglycoside	Matrix	Sample preparation	Sample clean-up	Recovery	Ref.
Ion-exchange SPE sorbents	GEN (C1, C1A, C2/C2A/C2B)	Fish tissue	2 g of sample → 10 mL 3% TCA with 0.4 mM EDTA → repetition of the procedure → dissolving of collected supernatants to 25 mL	LC-SCX ion-exchange SPE cartridges (3 mL/500 mg) - conditioning: 5 mL MeOH, 5 mL H_2O - sample volume: 12.5 mL - washing: 5 mL H_2O, 5 mL MeOH - elution: 5 mL MeOH with NH_3 (17:3 v/v)	80 – 110%	[24]
	SPC, STR, DHSTR, AMI, RIB, KAN, PAR, APR, GEN (C1, C1A, C2/C2A/C2B), NEO	Animal tissue (muscle), milk	*Sample pretreatment – muscle:* 3 g of sample → 15 mL (two steps: 10 + 5 mL) 10 mM NH_4Ac with 0.4 mM EDTA, 0.5%	Accell Plus CM, WCX SPE cartridges (6 mL/500 mg) - conditioning: 3 mL ACN, 6 mL H_2O - washing: 6 mL H_2O - elution: 3 mL 175 mM NH_4FA	72 – 96%	[29]

			NaCl and 2% TCA → pH adjustment to 6.5 → dilution to 50 mL *Sample pretreatment – milk:* 2 g of sample → 15 mL (two steps: 10 mL + 5 mL) 0.25% TCA → pH adjustment to 6.5 → dilution to 50 mL			
	STR, DHSTR, HYG, SPC, KAN, APR, GEN (C1, C2/C2A, C1A), NEO, TOB	Honey, royal jelly	5 g or 2 g of sample (respectively honey, royal jelly) → 0.25 mg trypsin → 5 mL H_2O → repetition of the extraction → collected supernatants filled up to 20 ml → splitting of the solution into 2 portions	Bond Elut Nexus WCX SPE cartridges (3 mL/150 mg) - 1st cartridge: - pH adjustment of 1 portion of ample to 7.5 - conditioning: 5 mL MeOH, 5 mL H_2O - washing: 7.5 mL H_2O - elution: 5 mL AA/ H_2O/ MeOH	75 – 114%	[14]

				(10:20:70 v/v/v/) Bond Elut Plexa PCX SPE cartridges (3 mL/150mg) - 2nd cartage - conditioning: 5 mL MeOH, 5 mL H_2O, 3 mL 20 mM HFBA - washing: 7.5 mL H_2O, 7.5 mL MeOH -elution: 5 mL NH_3/ H_2O/ MeOH (20:20:60 v/v/v)		
	HYG, AMI, KAN, RIB, APR, TOB, GEN, NEO	Animal feeds	1 g of sample → 5 mL 10 mM KH_2PO_4 with 0.4 mM EDTA and 2% TCA → repetition of the extraction → pH adjustment to 5.5	Oasis MCX SPE cartridges (3 mL/60 mg) - conditioning: 3 mL MeOH + 2% AA - washing: 3 mL 2% AA, 3 mL H_2O - elution: 5 mL MeOH + 20% NH_3	61 – 104%	[38]
Non-polar SPE sorbents	SPC, TOB, GEN, KAN, HYG, APR, STR, DHSTR, AMI,	Animal tissue (muscle),	10 g of sample (1 mL of milk)→ 0.25 mL 150 mM EDTA (0.05 mL for	C18 (DSPE) - mixing of sample and 25 mg of C18	37 – 98%	[3]

	NEO	milk	milk sample) → 10 mL 15% TCA (1 mL for milk sample)	sorbent		
	NEO, STR, DHSTR, GEN, KAN, SPC	Honey	5 g of sample →15 mL 0.1% HFBA	Strata-XL SPE cartridge (6 mL/200 mg) - conditioning: 6 mL MeOH, 6 mL H_2O, 6 mL 0.1% HFBA - washing: 6 mL 01% HFBA - elution: 5 mL ACN	87 – 127%	[33]
	VAL	Rice (rice straw, brown rice, rice hull)	5 g of sample → 20 mL MeOH/H_2O (9:1 v/v) (10 mL for brown rice) → 1 g NaCl	C18, GCB, PSA (DSPE) - sample volume: 1.5 ml - tube with sorbent (50 mg C18 – rice hull, 10 mg GCB and 40 mg PSA – rice straw, 10 mg GCB and 30 mg C18 – brown rice)	78 – 94%	[32]
Hydrophilic-lipophilic	APR, AMI, SPC, KAN, NEO, PAR,	Animal tissue (muscle, liver,	5 g of sample → 10 mL 5% TCA → repetition of	Oasis HLB SPE cartridges (3 mL/60 mg) – 2 steps procedure	47 – 93%	[23]

balance SPE sorbents	STR, DHSTR, TOB, GEN (C1, C2/C2A, C1A), HYG, SIS, NET	kidney)	the procedure → 5 mL 0.2 M HFBA → 5 mL n-hexane	- conditioning - 1st cartridge: 3 mL MeOH, 3 mL H_2O, 3 mL 0.2 M HFBA - sample volume - 1st cartridge: 5 mL - effluent pH adjustment to 8.5 - conditioning - 2nd cartridge: 3 mL MeOH, 3 mL H_2O, 3 mL 0.2 M HFBA, 3 mL solution of NaOH (pH 8.5) - sample volume - 2nd cartridge: all collected solution alter alkalization - connection of the 2 cartridges - washing: 5 mL H_2O - elution: 6 mL ACN with 0.15 M HFBA (4:1 v/v)		
	STR, TOB, NEO	Fish tissue	10 g of sample → 0.2 mL 150 mM EDTA → 10 mL 15% TCA → 10	Urea-formaldehyde monolithic cartridge for hydrophilic online in-tube SPME	82 – 97%	[25]

			mL n-hexane → pH adjustment to 7 → filtration → dilution of 5 mL of sample to 20 mL with ACN/H_2O (50:50 v/v) + 0.2% TFA	- sampling solution: ACN/H_2O (50:50 v/v) + 0.2% TFA - elution volume: 0.15 mL		
	SPC, DHSTR, AMI, KAN, TOB, APR	Honey, milk	*Sample pretreatment – honey:* 1 g of sample → 20 mL H_2O → pH adjustment to 5 *Sample pretreatment – milk:* 20 mL of sample → 1 mL TFA → dilution of 2 mL of sample to 20 mL with H_2O → pH adjustment to 5	Multiple monolithic poly(methacrylic acid-co- ethylenedimethacrylate) fibers (20 x 0.5 mm): - fibers activation: MeOH, H_2O - direct immersion of fiber into sample (40 min) - desorption: 0.4 mL H_2O/ACN/FA (94:5:1 v/v/v)	68 – 110%	[19]

	DHSTR, STR, KAN, SPC	Honey	2 g of sample → 10 mL 5 mM K_2HPO_4 (pH 11)	PVA-Sil SPE cartridge (3 mL/200 mg) - conditioning: 5 mL MeOH, 5 mL ACN/H_2O (90:10 v/v) - washing: 5 mL H_2O - elution: 2 mL H_2O/ACN/FA (90:9:1 v/v/v)	84 – 112%	[30]
	STR	Apples	5 g of sample → 20 mL of 10 mM KH_2PO_4 (pH 4) with 0.4 mM EDTA and 2% TCA → pH adjustment to 7.5	Oasis HLB SPE cartridges (6 mL/200 mg) - conditioning: 6 mL MeOH, 6 mL H_2O, - washing: 3 mL H_2O - elution: 6 mL MeOH + 3% FA	101 – 105%	[2]
MIP SPE sorbents and functionalized magnetic nanoparticles	AMI, APR, DHSTR, GEN (C1, C2/C2A, C1A), HYG, KAN, NEO, PAR, SIS, SPC, STR, TOB	Animal tissue (muscle, fat), fish, milk, egg – raw and processed	2 g of sample → 0.5 mL 0.5% EDTA → 20 mL 2% TCA → 4 mL 80 mM $(NH_4)_2CO_3$	SupelMIP SPE-AGs cartridges (3 mL/50 mg) - conditioning: 1 mL MeOH, 1 mL 50 mM K_3PO_4 (pH 7),	Approx. 90 – 100%	[27]

		food products		- sample volume: 3 mL - washing: 3 mL H_2O, 1 mL H_2O/ACN (6:4 v/v), 1 mL DCM/MeOH (50:50 v/v) - elution: 1 mL 30 mM HFBA in ACN/H_2O (25:75 v/v)		
	STR, DHSTR, KAN, GEN C1A, SPC, AMI, TOB, SIS, PAR, NET, HYG	Animal tissue (muscle), honey, milk	*Sample pretreatment – muscle and milk:* 2 g of sample → 5 mL 10 mM KH_2PO4 with 0.4 mM EDTA and 2% TCA → repetition of the procedure → dilution (1:1 v/v) with 50 mM K_3PO_4 → pH adjustment to 7 *Sample pretreatment – honey:*	SupelMIP SPE-AGs cartridges (3 mL/50 mg) - conditioning: 1 mL MeOH, 1 mL 50 mM K_3PO_4 (pH 7 ÷ 8.5), - washing: 3 mL H2O, 1 mL 0.1% NH_3 solution, 1 mL ACN/H_2O (40:60 v/v), 1 mL MeOH/DCM (50:50 v/v) - elution: 1 mL MeOH/H_2O (80:20 v/v) + 0.1% FA	78 – 95%	[28]

			2 g of sample → 10 mL 50 mM K_3PO_4 → pH adjustment to 7			
	AMI, APR, DHSTR, GEN (C1, C2/C2A, C1A), KAN, PAR, SPC, STR, TOB	Milk and milk-based food products	2 g of sample → 0.25 mL 15% TCA → 1 mL n-hexane → 3.5 mL 50 mM potassium phosphate (pH 7) → pH adjustment to 7	SupelMIP SPE-AGs cartridges (3 mL/50 mg) - conditioning: 1 mL MeOH, 1 mL 50 mM K_3PO_4 (pH 7), - sample volume: 3 mL - washing: 3 mL H_2O, 1 mL DCM/MeOH (50:50 v/v) - elution: 1 mL ACN/H_2O (20:80 v/v) + 1% FA with 20 mM HFBA	70 – 106%	[34]
	STR, DHSTR, KAN	Honey	2 g of sample → 10 mL ACN with 5 mM K_2HPO_4 (pH = 7) (10:90 v/v)	Magnetic Fe_3O_4@PVA nanoparticles for DSPE (40 mg) - conditioning: ultrasonification of sorbent in 5 mL of MeOH and washing with ACN/H_2O (10:90 v/v)	83 – 101%	[36]

				- washing: H_2O - elution: 1 mL H_2O/ACN/FA (80:19:1 v/v/v)		
	AMI, DHSTR, TOB, GEN (C1, C2/C2A, C1A)	Honey	0.2 g of sample → 2 mL H_2O	Magnetic Fe_3O_4@SiN- galactitol nanoparticles for DSPE (1 mg) - elution: 0.15 mL 190 mM NH_4FA (pH 3)	84 – 109%	[37]
	STR, KAN, APR, GEN, TOB, PAR	Environmental water	50 mL of sample	DMIPs SPE cartridges (2 mL/30 mg) - conditioning: 3 mL MeOH, 3 mL H_2O, - washing: 3 mL H_2O - elution: 3 mL H_2O + 1% FA	70.8 – 108.3%	[35]

305

306 *2.2 Separation and detection of aminoglycosides in LC- and CE-based analysis*

307 High polarity and polycationic character of AGs can cause a variety of problems during their
308 chromatographic separation in native forms. Additionally, considering possible ways of
309 detection, the absence of chromophoric or fluorogenic moieties complicates the determination
310 of these compounds. Direct AGs detection with UV or fluorescence detectors is not preferred,
311 although it has found some application in pharmaceutical formulations control [39,40]. Wider
312 use of this kind of detection for underivatized AGs does not seem to be possible due to the
313 high risk of possible matrix-related interferences. Furthermore, these methods cannot be used
314 for every AG. For example, it was shown that using direct UV detection amikacin and
315 tobramycin can be analysed, but in the case of gentamycin, it is not possible [40].

316 One way to deal with this problem is derivatization. Not only does it make detection
317 easier, but also facilitates chromatographic separation of analytes by lowering their polarity.
318 Derivatization products usually can be easily separated under reversed-phase conditions. The
319 most popular derivatization agents for AGs are: 1-naphthyl isothiocyanate (NITC), 9-
320 fluorenylmethyloxycarbonyl (FMOC), 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate
321 (AQC), o-phthalaldehyde (OPA), and 1-fluoro-2,4-dinitrobenzene (FDNB) [15,16,20,41,42].
322 It has to be stressed however, that the selection of derivatization agent can be a difficult task.
323 Some derivatizing agents (e.g. FMOC) can react with primary and secondary amino groups of
324 AGs with different selectivity and efficiency, thus producing multiple derivatives from a
325 single analyte (Fig. 4). This, in turn, will impair quantitation and increase matrix effects levels.
326 The stability of derivatization products is another source of potential issues. For instance,
327 OPA is recommended to be used in post-column mode due to low stability of its derivatives
328 [43,44]. Certain derivatization agents call for quite harsh reaction conditions, such as high
329 temperatures (e.g. derivatization with FDNB requires around 85°C) which in some scenarios
330 may be a limiting factor [42]. What is more, the efficiency of derivatization highly depends
331 on the composition of the sample matrix. Overall, methods involving derivatization are more
332 commonly used to analyse samples with less complex matrices (e.g. pharmaceuticals) or
333 when a single AG is to be determined [15,16,20]. It seems that the application of
334 derivatization reactions in combination with reversed-phase separation conditions is
335 becoming less prominent.

336 The latest trends in AGs determination focus on developing protocols employing
337 hydrophilic interaction (HILIC) or ion-pairing liquid chromatography (IPLC). These

approaches allow the determination of AGs in their native forms but also impose the use of a different set of detection techniques, like mass spectrometry (single or tandem MS), evaporative light scattering detection (ELSD), charged aerosol detection (CAD) or pulsed amperometric detection (PAD). Table 4 summarizes exemplary HPLC methodologies for AGs analysis. The referenced publications are focused mainly on IPLC and HILIC.

When developing a method for the determination of aminoglycosides which involves the use of a separation technique such as LC or capillary electrophoresis discussed in Section 3 of this review, it is also important to carefully consider the choice of the detector. In the case of the determination of single analyte and analysis of less complex matrices detectors such as ELSD, UV, FLD or CAD could be cost-effective. However, when the opposite is true, or when the analytes are present in the sample at trace concentration levels, tandem mass spectrometry seems to be the method of choice. The application areas and detection levels of various detectors coupled with separation techniques for the determination of AGs are listed in Table 3.

Table 3. Detectors most commonly used for determination of aminoglycosides in conjunction with separation techniques.

Type of detector	Tentative detection levels	Remarks
Refractive index detector (RID)	100 000 ng/mL	- determination of aminoglycosides in the native form; - low sensitivity; - nonselective; - response varies with temperature and mobile phase composition - isocratic elution is preferred, which hinders separation of analyte mixtures; - currently, the use for analysis of AGs is marginal;
Evaporative light scattering detector (ELSD)	100 ng/mL	- determination of aminoglycosides in the native form; - higher sensitivity compared to RID and stable baseline during gradient elution; - nonselective; - nonlinear response; - possible problems with detection under HILIC conditions (high concentration of buffers may suppress the signal).
Charged aerosol detection (CAD)	100 ng/mL	- determination of aminoglycosides in the native form; - better sensitivity compared to some universal detectors, such as RID; - narrow range of linearity; - sensitivity dependent on the content of organic solvent in the mobile phase; - possible problems with detection under HILIC conditions (high concentration of buffers may suppress the signal).
Diode array detection (DAD)	10 000 ng/mL (with	- in most cases derivatization of analytes is required; - better selectivity compared to RID and ELSD;

	derivatization) >10 000 ng/mL (without derivatization)	- wide range of linear response; - high reliability; - used with both LC and CE.
Fluorescence detection (FLD)	100 ng/mL	- derivatization of analytes is required; - improved sensitivity and selectivity compared to DAD); - used with both LC and CE.
Tandem mass spectrometry (MS/MS)	1 ng/mL	- determination of aminoglycosides in the native form; - highly sensitive and selective detection; - the most powerful tool for the simultaneous determination of multi-component mixtures; - high concentration of buffers (HILIC) and some mobile phase additives (IPLC) negatively affects sensitivity; - most commonly used with LC, can be used with CE.
Amperometric detectors	500 ng/mL	- determination of AGs in the native form; - highly sensitive detection, however in the case of samples with complicated matrices problems with repeatability may occur due to deterioration of of electrodes; - highly alkaline pH is necessary (pH > 11) – anion exchange mode; - used with both LC and CE.
Capacitively coupled contactless conductivity detection (C^4D)	10 ng/mL	- determination of AGs in the native form; - universal type of detection suitable for miniaturization and coupling with CE systems; - good sensitivity; - issues with baseline stability due to changes in the conductivity of the background electrolyte; - most commonly used with CE.
laser-induced fluorescence detection (LIF)	10 ng/mL	- derivatization of aminoglycosides necessary; - high sensitivity and selectivity; - most commonly used with CE.

Ion-pairing liquid chromatography (IPLC)

Due to the presence of multiple amino groups in the structures of AGs molecules, they tend to form polyvalent cations in the solution. Such cations can interact with negatively charged moieties, e.g. anions of perfluorinated organic acids. The resulting ion pairs are notably less polar than AGs, as described in section 2.1. This phenomenon can be taken advantage of during LC separation to increase AGs retention and separation selectivity using LC columns packed with non-polar sorbents (e.g. C8, C18, etc.). Main drawbacks of using IPLC include the diminished lifetime of chromatographic equipment and columns as well as detection problems when mass spectrometry is employed (ion suppression). Despite some disadvantages, IPLC finds widespread application due to good separation of AGs in multi-component mixtures (competitive to other LC-based techniques). Problems with column lifetime may be alleviated using their dedicated versions showing higher resistance against low pH conditions.

The most commonly used ion-pairing (IP) reagents which provide the best AGs separation are volatile perfluorinated organic acids, for example, heptafluorobutyric acid (HFBA), pentafluoropropionic acid (PFPA) or trifluoroacetic acid (TFA). IP agents are added to the mobile phase in concentrations ranging from 0.1% to 1% (v/v) [10,11,33,45,46]. Non-volatile ion-pairing reagents (e.g. alkylsulfonic acids) are not used in modern analytical chemistry due to their incompatibility with the majority of detectors used for AGs analysis (MS, evaporative light scattering (ELSD), charged aerosol detectors (CAD)).

Depending on the complexity of the sample matrix, different detectors may be used for AGs determinations using IPCL. Usually, for well-defined and relatively simple matrices such as pharmaceutical formulations, (ELSD) and (CAD) [10,11,13] are a good choice. Their advantages are good sensitivity (LODs start from around 10 ng injected on column), a similar response to all AGs, simple and rugged construction and low running costs. The downsides are lack of selectivity and non-linear (sigmoidal) response curve. While the non-linearity problem can be solved quite easily with modern computer technology, the lack of selectivity calls for the use of more elaborate sample preparation protocols. The performance of IPLC methods can be significantly improved by employing tandem mass spectrometry at the detection stage [3,21,23,26,27,46]. Higher sensitivities and sample throughput, better selectivity and multi-residue analysis capability can be obtained at the expense of higher instrumentation, maintenance and personnel costs. In many cases, the use of mass spectrometric detection allows for reliable determination of analytes despite their non-baseline separation. The choice of the ion-pairing reagent in IPLC-MS/MS should be carefully considered. While TFA was successfully used to determine 15 AGs [26], it is commonly regarded as a troublemaker in the context of mass spectrometry. Pentafluoropropionic acid (PFPA) could be used as an alternative, as it causes less ion suppression and in comparison to HFBA it has a lower affinity to the stationary phase, however, its superiority over HFBA is yet to be clearly demonstrated [46].

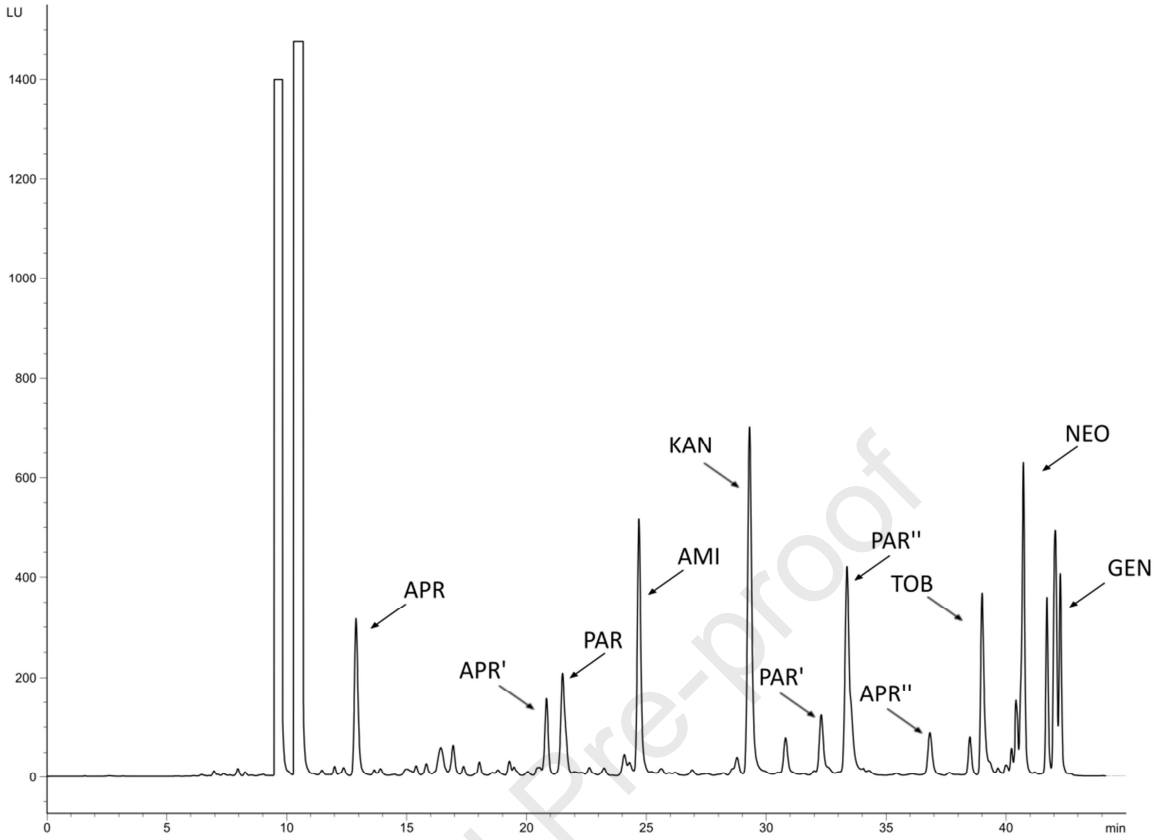

Fig. 4. Example of chromatogram after derivatization of AGs mixture with FMOC; APR – apramycin, PAR – paromomycin, AMI – amikacin, KAN – kanamycin, TOB – tobramycin, NEO – neomycin, GEN – gentamycin.

Hydrophilic interaction liquid chromatography (HILIC)

Hydrophilic interaction liquid chromatography can be used when the separation of polar, water-soluble chemical compounds such as aminoglycoside antibiotics is desired. It is believed that the separation mechanism relies on interactions between analytes and quasi-stationary phase consisting of water-rich layer surrounding proper stationary phase particles. Analyte's retention behaviour is rather difficult to predict since it is dependent on several factors including the composition of the mobile phase (percentage and kind of organic component), the character of stationary phase, pH, type and concentration of buffer, temperature and so on.

Stationary phases used in HILIC include bare, diol, pentafluorophenyl, cyanopropyl or amino-modified silica gels. These phases can be successfully used for separation of various classes of compounds, although for AGs much better results are frequently obtained with

novel stationary phases (e.g. ZIC-HILIC and its variants) developed specifically for HILIC separations (Fig. 5). ZIC-HILIC stationary phases combine hydrophilic partitioning with weak ionic interactions resulting from the presence of zwitterionic (sulfobetaine, phosphorylcholine) groups covalently bound to the surface of silica particles. Phosphorylcholine-modified silica gels seem to be well suited for AGs separations [47].

Regarding the aqueous mobile phase composition, typically buffers with a concentration lower than 60 mM (or water acidified with e.g. formic acid) are preferred for the determination of no more than 5 AGs in the sample [2,23,30,36]. In these conditions, baseline separation of AGs is extremely difficult to achieve. The separation resolution increases with the concentration of the buffer. To obtain satisfactory separation for simultaneous multi-AGs mixture, buffer concentrations higher than 150 mM are needed [22,28,31,34]. What is more, most of the protocols include the addition of 0.05 – 2% (v/v) FA to the mobile phase (both to the buffer and the organic components) [19,22,28,30,31,34] which improves peak shape (especially their characteristic "tailing" under HILIC conditions) through the weak ionic interaction with amino groups (affecting slightly reduction of AGs interaction with the stationary phase).

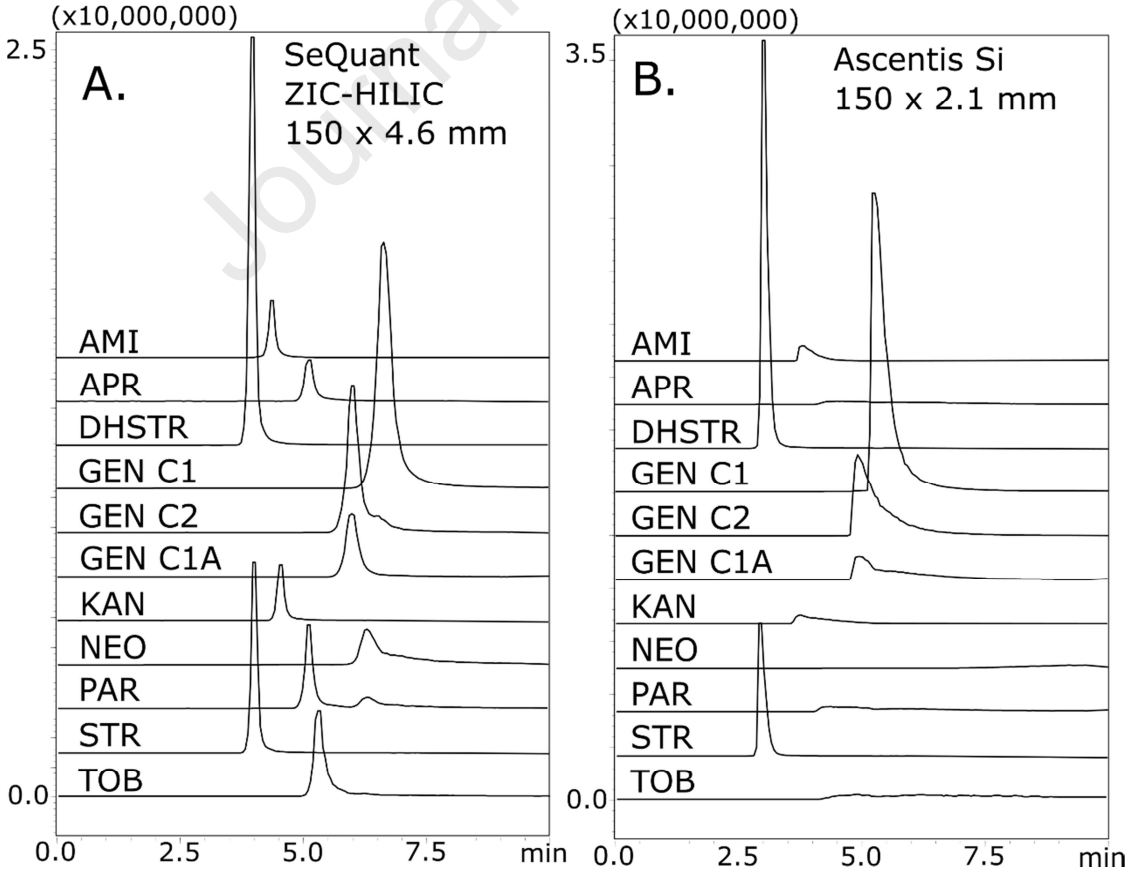

Fig. 5. Comparison of chromatographic separation of 11 AGs under HILIC conditions using SeQuant ZIC-HILIC (A) and Ascentis Si (B) LC columns; AMI – amikacin, APR – apramycin, DHSTR – dihydrostreptomycin, GEN – gentamycin, KAN – kanamycin, NEO – neomycin, PAR – paromomycin, STR – streptomycin, TOB – tobramycin.

Baseline separation of multiple (more than 8) AGs under the HILIC conditions is difficult if possible at all, as illustrated by an attempt to simultaneously determine 9 AGs using HILIC-ELSD [12]. Despite long chromatographic run (120 min), baseline separation of analytes could not be obtained. Authors tried to resolve overlapping peaks using 2D-LC with IPLC as a 2^{nd} separation dimension but this attempt was only partially successful, and the total separation time was 240 min.

These limitations can be overcome by using HILIC in combination with mass spectrometric detection since it does not require the use of problematic mobile phase additives (e.g. perfluorinated IP reagents). Mass spectrometric detection in general, and tandem mass spectrometry in particular, due to its selectivity allows to overcome the problem of non-baseline separation of analytes and offers significantly shorter run times (e.g. 9 min when fast HILIC-MS/MS was applied for the determination of 11 AGs in milk-based food products [34]). Attention should be devoted to the effect of the composition of the mobile phase on the peaks shape and the detector's response, with variables such as the impact of the buffer or the organic modifier (notably, methanol was found to be superior to the commonly used acetonitrile in certain applications [28]). ZIC-HILIC columns are commonly used to achieve acceptable separation of AGs and were shown to perform better than another type of zwitterionic column, namely Obelisc R. The stationary phase of the latter is characterized by the presence of carboxyl acid functional groups instead of sulfonic groups present in the ZIC-HILIC columns [28]. Another approach would be to use graphitized LC columns [24], however, they are characterized by some limitations such as fluctuations in analytes retention times and time-consuming column deactivation step. In a study in which 3 different HILIC-ESI conditions for multiresidue drugs determination, including 11 AGs and colistins in animal muscle and milk samples were compared it was concluded that with ZIC-HILIC column, the symmetry and retention of neomycin peak weren't acceptable. On the other hand, problems with retention of spectinomycin and neomycin were observed with a graphitized column. As the most suitable solution, the authors have chosen bare silica Poroshell HILIC column [29].

457 Table 4. HPLC methods used for determination of aminoglycoside antibiotics in various matrices.

LC conditions	Aminoglycoside	Matrix	LC column	Mobile phase	Detection	LOD/LOQ	Ref.
RP	GEN, NEO	Animal tissues (kidney, liver, muscles)	Hypersil BDS C18 (100 x 4.6 mm), 5µm	ACN/H_2O (85:15 v/v)	$FLD_{FMOC-Cl}$	LOD: 0.05 – 0.10 µg/g	[20]
	STR, DHSTR, HYG, SPC, KAN, APR, GEN (C1, C2/C2A, C1A), NEO, TOB	Honey, royal jelly	Porshell 120 EC-C8 (100 x 2.1 mm), 2.7 µm	A: 0.5 mM NH_4Ac + 0.1% FA B: ACN + 0.1% FA	ESI-MS/MS	LOD: 0.005 – 0.0125 µg/g LOQ: 0.001 – 0.025 µg/g	[14]
IPLC	HYG, AMI, KAN, RIB, APR, TOB, GEN, NEO	Animal feeds	Hypersil BDS C18 (250 x 4.6 mm), 5 µm	A: ACN/H_2O (5:95 v/v) + 20 mM HFBA B: ACN/H_2O (50:50 v/v) + 20 mM HFBA	ELSD	LOD: 0.2– 0.7 µg/g	[38]
	APR, AMI, SPC, KAN, NEO, PAR,	Animal tissue (muscle, liver,	Atlantis dC18 (150 x 2.1 mm), 5 µm	A: ACN + 20 mM HFBA	ESI-MS/MS	LOD: 0.0009 – 0.009 µg/g	[23]

	STR, DHSTR, TOB, GEN (C1, C2/C2A, C1A HYG, SIS, NET	kidney)		C: ACN/H_2O (5:95 v/v) + 20 mM HFBA D: ACN/H_2O (50:50 v/v) + 20 mM HFBA		LOQ: 0.003 – 0.030 µg/g	
	STR, TOB, NEO	Fish tissue	Syncronis C18 (250 x 4.6 mm), 5 µm	H_2O + 0.2% TFA with ACN (9:1 v/v)	ELSD	LOD: 0.0035 – 0.0052 µg/g	[25]
	AMI, APR, DHSTR, GEN (C1, C2/C2A, C1A), HYG, KAN, NEO, PAR, SIS, SPC, STR, TOB	Animal tissue (muscle, fat), fish, milk, egg – raw and processed food products	Kinetex C18 (100 x 2.1 mm), 2.6 µm	A: 20 mM HFBA B: ACN	MS/MS	-	[27]
	SPC, TOB, GEN, KAN, HYG, APR, STR, DHSTR, AMI, NEO	Animal tissue (muscle), milk	Waters X-Terra C18 (100 x 2.1 mm), 5 µm	A: 10 mM NFPA B: ACN + 10 mM NFPA	ESI-MS/MS	LOD: 0.005 – 0.100 µg/g LOQ: 0.0125 – 0.250 µg/g	[3]

	NEO, STR, DHSTR, GEN, KAN, SPC	Honey	Kinetex XB C-18 (100 x 3 mm), 2.7 µm	A: H_2O + 0.1% HFBA B: ACN	ESI-MS/MS	LOQ: 0.005 – 0.075 µg/g	[33]
2D-LC HILIC x IPLC	SPC, BAC, DHSTR, STR, GEN, KAN, AMI, APR, PAR, NEO	Tablets	1st dimension: Grom-Sil 120 Diol (250 x 4.6 mm), 5 µm	A: 20 mM NH_4FA (pH 2.5) B: ACN	ELSD	LOD: 130 µg/mL LOQ: 240 µg/mL	[12]
			2nd dimension: Luna C18 (250 x 2.1 mm), 5 µm	A: 5 mM PFOA/ACN (95:5 v/v) B: 5 mM PFOA/ACN (5:95 v/v)			
HILIC	SPC, STR, DHSTR, AMI, RIB, KAN, PAR, APR, GEN (C1, C1A, C2/C2A/C2B), NEO	Animal tissue (muscle), milk	Poroshell 120 HILIC (100 x 2.1 mm), 2.7 µm	A: 1 mM NH_4FA + 1% FA B: ACN	HESI-II-Q-Orbitrap	LOD: ≤ 0.033 µg/g	[29]

	AMI, APR, DHSTR, GEN (C1, C2/C2A, C1A), KAN, PAR, SPC, STR, TOB	Milk and milk-based food products	Kinetex HILIC (100 x 2.1 mm), 1.7 µm	A: 150 mM NH_4Ac + 0.1% FA B: ACN	ESI-MS/MS	LOQ: 0.0042 – 0.049 µg/g	[34]
	SPC, DHSTR, AMI, KAN, TOB, APR	Honey, milk	ClickXlon HILIC (150 x 3 mm), 5 µm	60 mM NH_4FA and ACN (90:10 v/v) + 1% FA	ESI-MS/MS	LOD: 0.0001 – 0.00059 µg/g	[19]
	DHSTR, STR, KAN, SPC	Honey	TE-Cys HILIC (150 x 3 mm), 3 µm	A: 30 mM NH_4FA + 1% FA B: ACN/H_2O (80:20 v/v) + 1% FA	ESI-MS/MS	LOQ: 0.0078 – 0.0194 µg/mL	[30]
	AMI, DHSTR, TOB, GEN (C1, C2/C2A, C1A)	Honey	Acclaim Mixed-Mode HILIC-1120 Å (150 x 4.6 mm), 5 µm	H_2O/ACN (80:20 v/v) + 0.1% FA	ESI-MS/MS	LOQ: 0.002 – 0.019 µg/g	[37]
ZIC-HILIC	SPC, STR, DHSTR, KAN, GEN (C1, C2/C2A, C1A),	Animal tissue (muscle), milk	SeQuant ZIC-cHILIC (100 x 2.1 mm), 3 µm	A: 200 mM NH_4Ac + 2% FA B: ACN + 2% FA	ESI-MS/MS *with post-column	LOQ: 0.00019 – 0.0025	[31]

	NEO, AMI				reagent (MeOH and NaAc)	µg/mL	
	STR, DHSTR, KAN, GEN C1A, SPC, AMI, TOB, SIS, PAR, NET, HYG	Animal tissue (muscle), honey, milk	ZIC-HILIC (50 x 2.1 mm), 3.5 µm	A: 175 mM NH_4FA + 0.3% FA B: MeOH + 0.3% FA	ESI-MS/MS	LOD: 0.002 – 0.030 µg/g LOQ: 0.007 – 0.100 µg/g	[28]
HypC(HILIC)	GEN (C1, C1A, C2/C2A/C2B)	Fish tissue	Hypercarb (100 x 2.1 mm), 5 µm	A: 5% NH_4OH B: ACN	ESI-MS/MS	LOD: 0.010 – 0.020 µg/g	[24]

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3. Alternative analytical techniques used in the determination of aminoglycosides

Liquid chromatography remains the gold standard in the determination of aminoglycosides in a variety of matrices. However, its application often entails the use of relatively expensive instruments and engagement of highly-trained personnel. While the same is true for certain capillary electrophoresis-based methods, such as CE-ESI-MS/MS, other emerging techniques, such as the use of immunoassays and microfluidic devices, could facilitate the development of less expensive, portable tests. These are unlikely to match the capabilities and versatility of the established LC-based methods in the near future, they could, however, greatly increase the access to AGs analyses, particularly in the farming and food processing industry and in resource-scarce settings.

Capillary electrophoresis

Aminoglycosides tend to form complexes and ionic species in aqueous solutions. This makes them suitable for electrophoretic separations. Capillary electrophoresis (CE) is a powerful separation technique which has been successfully used for determination of this class of compounds. High resolving power is the most important advantage of CE, affordability of instrumentation and relatively short separation times being less important. The biggest challenge in the use of CE is improving selectivity and detection sensitivity. In many cases combining the high resolving power with sensitive and selective detection (e.g. MS) is difficult or not possible at all due to incompatibility of non-volatile buffers used in CE with mass spectrometry. Furthermore, due to the very short lifetime of the columns (Huidobro et al. [48] suggested that the capillary should be changed after just 8 runs to ensure reproducible results) seriously limits the sample throughput.

Capillary electrophoresis and liquid chromatography share almost the same set of detectors, and therefore the same detection problems stemming from AGs properties are reported in the case of CE. The solutions to these problems are also similar. Derivatization with o-phthalaldehyde (OPA) allows the use of FLD detection [49]. Another reagent, 6-carboxyfluorescein succinidyl ester (CFSE) can be used to detect kanamycin, bekanamycin and paromomycin using laser-induced fluorescence detection (LIF) [50]. Direct UV detection at 195 ± 5 nm without [48] or after complexation with borates [51] as well as indirect UV detection for the determination of AGs have been described [52]. However, the use of CE coupled with both direct and indirect UV detection plays a marginal role in the determination

of underivatized AGs due to relatively low sensitivity (LOD > 10 μg/mL) of this detection technique [48,51–53].

In order to analyse underivatized AGs with higher sensitivity, other types of detectors have to be used, such as electrochemical detectors, in which case amperometric detectors (AD) with transition metal electrodes like Cu and Ni are used [54,55]. The main problem associated with CE-AD methods in AGs analysis is electrode fouling [56]. In general, due to poor Ni-electrodes stability, the use of Cu-based electrodes is preferred. Various electrode modifications have been proposed to further improve CE-AD performance [56–58], e.g. the use of Chemically modified copper electrode (Cu microparticle-modified carbon fiber microdisk array electrode, Cu-CFE) which produced repeatable results and LOD values in the low microgram per millilitre range [59]. Very good results (LOD = 10 ng/mL) were also reported by Mukhtar et al. [60] in a study in which CE was coupled with capacitively coupled contactless conductivity detection (C^4D) to determine tobramycin in human plasma. The popularity of CE-C^4D methods is currently growing, mainly due to their flexibility as well as comprehensive nature towards all ionic analytes [61].

The improvement of the capabilities of the analytical instrumentation in the past two decades drastically increased the application potential of CE in the determination of AGs. One of the most promising approaches is the coupling of CE with highly-selective and sensitive mass spectrometric detectors [62]. However, while it is possible in general, this solution does not seem to be practical, at least at the moment. As already mentioned, bringing out the full potential of CE requires the use of non-volatile buffers which are incompatible with mass spectrometry. Although the replacement of the said buffers with their volatile alternatives is possible, the resulting methodology would suffer either from low resolution or low sensitivity. Up to now, only two reports describing the successful determination of AGs using CE-MS/MS instrumentation were published [63,64].

Alternatively, CE sensitivity could be improved by using various online pre-concentration techniques such as field-enhanced sample injection (FESI) or field-amplified sample stacking (FASS) [49,55]. Long et al. [49] compared results of kanamycin determination (UV detection) with and without online FASS pre-concentration. The method sensitivity was twenty times higher using FASS. Ge et al. [55] used hyphenation of transient moving substitution boundary (MSB) with FESI for streptomycin, neomycin and kanamycin determination (AD detection). Application of online pre-concentration techniques improved

522 the sensitivity of the method by two to three orders of magnitude over those of previously
523 reported CE-AD methods. CE methods for the determination of aminoglycoside antibiotics
524 are summarized in Table 5.

525 Table 5. CE methods for the determination of aminoglycoside antibiotics.

Method	Aminoglycoside	Matrix	Sample preparation	CE conditions	LOD/LOQ Rec.	Ref.
CE-UV_{OPA}/ FASS CE-UV_{OPA}	KAN	Human serum	WCX SPE; Pre-capillary derivatization with OPA and mercaptoacetic acid	Background buffer: 30 mM borax + 16% v/v MeOH (pH = 10) Capillary: uncoated fused-silica (42.5 cm x 50 μm ID) Separation voltage: 23.5 kV Temperature: 20°C	CE-UV_{OPA} LOD: 2 μg/mL Rec. 90% FASS CE-UV_{OPA}: LOD: 0.1 μg/mL Rec. 60%	[49]
CE-Argon ion LIF_{CFSE}	KAN, BEK, TOB, PAR	Human plasma	Protein precipitation (ACN); Pre-capillary derivatization with CFSE	Background buffer: 30 mM sodium borate buffer (pH = 9) Capillary: fused silica capillary (50 cm x 50 μm ID)	LOD: 7 – 14 ng/mL Rec. 92 – 105%	[50]

				Separation voltage: 8 kV Temperature: N/A		
CE-UV_{direct}	STR, DHSTR	Standard solution	-	a) Option 1 – anodic mode: Background buffer: 160 mM sodium tetraborate buffer (pH = 9) Capillary: uncoated fused silica (90 cm x 50 μm ID) Separation voltage: 18 kV Temperature: 34°C b) Option 2 – cathodic mode: Background buffer: 75 mM sodium tetraborate buffer and 0.5 mM myristyltrimethylamonium bromide (TTAB) (pH = 9) Capillary: uncoated fused	LOD: 10 μg/mL	[51]

				silica (90 cm x 50 μm ID) Current: – 18 kV Temperature: 34°C		
CZE-UV_{direct}	NEO	Pharmaceutical formulations (ointments)	LLE with chloroform	Background buffer: 35 mM orto-phosphoric acid + 15 mM acetic acid (pH = 4.7); Capillary: polyacrylamide (30 cm x 50 μm ID) Separation voltage: 20 kV Temperature: 25°C	Rec. 99.93%	[48]
CZE-$UV_{indirect}$	NEO, DHSTR, LIV, AMI, KAN, TOB, SIS	Pharmaceutical formulations (ear drops), standard solution	Addition of cetyltrimethylammonium bromide	Background buffer: 0.01 M imidazole acetate + Fluorad® FC 135 (pH = 5) Capillary: fused silica (67 cm x 50 μm ID) Separation voltage: 12.5 kV	LOD: 10 – 50 μg/mL	[52]

				Temperature: N/A		
CE-AD	NET, TOB, LIN, KAN, AMI	Pharmaceutical formulation (injections)	-	Background buffer: 125 mM NaOH Capillary: uncoated fused silica capillary (45 cm x 50 μm ID) Separation voltage: 6.2 kV Temperature: N/A	LOD: 0.63 – 2.7 μg/mL Rec. 91 – 99%	[59]
FESI-MSB with CE-AD	STR, NEO, KAN	River water	C18 SPE Addition of 18-crown-6-tetracarboxylic acid (18C6H4)	Background buffer: 15 mM sodium tetraborate buffer + 55 mM NaOH + 10% ACN Concentration of 18C6H4 in pseudostationary phase: 150 mM Capillary: fused-silica (75 cm x 25 μm ID) Separation voltage: 17 kV	LOD: 0.35 – 4.3 ng/mL Rec. 87.7 – 106.3%	[55]

				Temperature: 25°C		
CE-C^4D	TOB	Human plasma	Dynamic mixed matrix membrane tip extraction	Background buffer: 200 mM acetic acid Capillary: fused base silica (55 cm x 50 μm ID) Separation voltage: 25 kV Temperature: N/A	LOD: 10 ng/mL Rec. 99.6 – 99.9%	[60]
CE-C^4D	AMI	Bronchial epithelial lining fluid	Addition of urease	Background buffer: 30 mM malic acid + 10 mM 18-Crown-6 + L-arginine (pH = 4.1) Capillary: fused base silica (65 cm x 75 μm ID) Separation voltage: 30 kV Temperature: 25°C	LOD: 0.14 μg/mL Rec. 100%	[65]
Sheathless CE-ESI-	AMI, PAR, HYG, APR, GEN C1	Milk	Protein precipitation (TCA, NaCl, EDTA,	Background buffer: 10% v/v acetic acid	LOQ: 0.67 μg/kg Rec. 76.2 –	[63]

MS/MS			NH_4Ac) PWCX-SPE	Capillary: bare fused silica (90 cm x 30 μm ID) Separation voltage: 25 kV Temperature: 25°C	110.0%	
CE-ESI-MS/MS	GEN (C1, C1A, C2), NEO, APR, PAR, DHSTR, SPC, STR	Honey	MIP-SPE	Background buffer: 200 mM formic acid + 7 mM ammonium hydroxide (pH = 2.2) Capillary: bare fused-silica (90 cm x 50 μm ID) Separation voltage: 25 kV Temperature: 25°C	LOD: 0.4 – 28.5 μg/kg Rec. 88.2 – 99.8%	[64]

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Immunological methods

Immunological methods, e.g. fluoroimmunoassay (FIA) [28], radioimmunoassay (RIA) [66], as well as enzyme-linked immunosorbent assay (ELISA) [67,68], are characterized by high sensitivity and LOD values in the range of few ng/mL, or even pg/mL when used for the determination of AGs. However, due to their high sensitivity, false-positive results are frequently obtained [69]. Additionally, low reproducibility of these methods makes them suitable only for screening and semi-quantitative testing, which usually needs confirmation by LC measurements [70]. Currently, the most often used immunological method in AGs analytics is competitive ELISA, which can be realized as either indirect or direct competitive ELISA. In both cases, the product of the enzymatic reaction is detected by spectrophotometric, fluorescence or chemiluminescence measurement.

Direct competitive ELISA (DC-ELISA) procedures are usually less labour- and resource-intensive and time-consuming than their IC-ELISA equivalents, however at the cost of higher false-negative results and no signal amplification. For example, DC-ELISA method for neomycin determination proposed by Jin et al. [71] guaranteed LOD = 2.73 ng/mL (for milk samples). In comparison, the equivalent IC-ELISA method developed by Xu et al. [67] was characterized by LOD = 0.08 ng/mL. There are cases, however, when DC-ELISA methods can be improved and perform at the same level as IC-ELISA ones. Jiang et al. [72] used horseradish peroxide-modified gold nanoparticles for determination of kanamycin and tobramycin in milk. The LOD of the method was improved fivefold over the conventional DC-ELISA.

In general, the ELISA procedures are very sensitive and relatively easy to carry out, even by untrained personnel, while the biggest problem associated with AGs determination using this technique is the cross-reactivity (CR) phenomenon resulting in poor selectivity of the assay [73]. To avoid cross-reactivity, highly specific antibodies are required, which makes the implementation of ELISA more expensive, complicated and time-consuming [74]. At present researchers working with both IC-ELISA and DC-ELISA are focused on the preparation of highly-selective antibodies to avoid or attenuate cross-reactivity of AGs. Examples of ELISA-based methods for determination of AGs are provided in Table 6.

557 Table 6. ELISA-based methods for the determination of aminoglycoside antibiotics.

Aminoglycoside	Matrix	Sample preparation	Enzyme	Substrate	Antibody	LOD/LOQ Rec.	Ref.
NEO	Milk	Protein precipitation (TCA)	HRP	TMB	Polyclonal	LOD: 0.08 ng/mL Rec. 85 – 110%	[67]
AMI	Milk	Protein precipitation (TCA)	HRP	TMB/H_2O_2	Polyclonal	LOD: 11.3 ng/mL Rec. 69.8 – 93.9%	[70]
KAN	Milk, honey	Milk: centrifugation, addition of $Na_2[Fe(CN)_5NO]\cdot H_2O$ and $ZnSO_4$ and deproteinization by centrifugation Honey: fat removing (PBS extraction)	SA-HRP	TMB	Polyclonal	LOD: 0.07 ng/mL Rec. 91.0 – 103.3%	[75]
NEO	Rabbit plasma,	-	HRP	OPD	Monoclonal	LOD: 2.73 – 6.85	[71]

	milk					ng/mL Rec. 87 – 108%	
NEO	Pig muscle, chicken muscle, egg, fish, milk, kidney	PBS extraction	HRP	TMB	Polyclonal	LOD: 0.1 – 20 μg/kg Rec. 75.1 – 105.8%	[68]
KAN, TOB	Milk	-	a) HRP b) AuNPs/ HRP	TMB	Monoclonal	a) 0.13 ng/mL b) 0.022 ng/mL Rec. 81 – 123.9%	[72]

HRP – horseradish peroxidase; TMB – 3,39,5,59-tetramethylbenzidine; SA-HPR – streptavidin-horseradish peroxidase; PBS – phosphate-buffered saline; OPD – o-phenylenediamine.

Spectrophotometric & spectrofluorimetric methods

Spectrophotometric and spectrofluorimetric methods can be used for the straightforward and non-separative analysis of AGs. Due to the lack of selectivity, the application of these methods is limited to the routine control of a single compound in e.g. pharmaceuticals formulations. In general, analytical procedures include a derivatization step with various reagents.

Although the vast majority of the spectroscopic methods used for AGs determination relies on derivatization, there are few exceptions, such as the use of direct fluorimetry for quick determination of apramycin in pharmaceuticals and milk samples [76]. Micelle-enhanced native apramycin fluorescence allowed to obtain sensitivity comparable with methods in which derivatization was used. Ghodake et al. [77] used silver nanoparticle (AgNP) probe coated with gallic acid for colourimetric determination of streptomycin in water, serum and milk samples with LOD lower than 0.1 ng/mL. Ma et al. [78] determined the tobramycin in milk and eggs samples with detection limits of 11 ng/mL using golden nanoparticle (AuNP)/single-stranded DNA-based colourimetric sensors. Recently, methods employing fluorescent quantum dots (QD) sensing for AGs determination have been developed. Some applications highlight the potential of QD-based procedures in selective determination of particular AGs in relatively complex matrices, following MIP SPE extraction (see Fig. 6) [79,80]. Furthermore, the development of miniaturized, AGs-specific biosensors could be particularly useful in system automation, enabling on-line detection of contaminants. Tang et al. [45] developed an evanescent wave aptasensor based on target binding facilitated fluorescence quenching (FQ-EWA) for such purpose. The selectivity towards a particular AG (kanamycin) was achieved using a fluorophore-labelled DNA aptamer. Notably, the FQ-EWA was characterised by relatively high durability, enabling more than 60 detection-regeneration cycles which showcases the application potential of AGs-specific biosensors, including electrochemical sensors, as discussed in the following sub-section on electrochemical methods.

The colourimetric methods have potential to be developed into point-of-need methods owing to the ongoing efforts to use them in conjunction with the ubiquitous smartphones, which combine a convenient interface, detector (CCD camera), processing power and network connectivity [81]. Such developments could greatly decrease the cost and increase the

availability of in-field AGs analysis. Examples of spectrophotometric and spectrofluorimetric methods for determination of AGs are shown in Table 7.

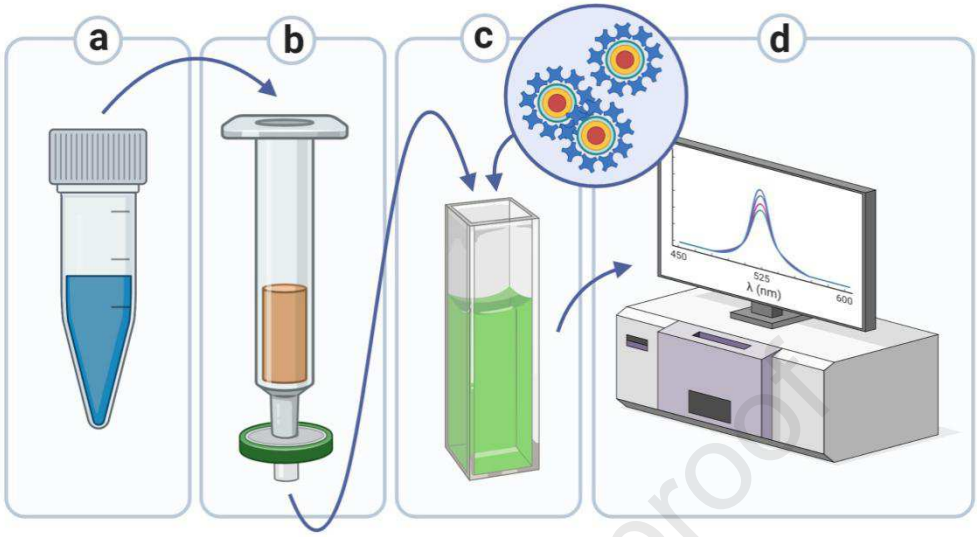

Fig. 6. Determination of kanamycin in vaccine samples based on its effect on the thioglycolic acid-CdTe quantum dots photoluminescence [79]. Aliquots of reconstituted vaccine samples (a) were loaded into an SPE cartridge coupled with a syringe filter and packed with kanamycin-MIP (b). After washing and elution with acidic water, the aliquots were added to a TGA-CdTe quantum dots dispersion probe (c) and the subsequent photoluminescence measurements were carried out using a luminescence spectrophotometer (d). Created with BioRender.com.

601 Table 7. Spectrophotometric and spectrofluorimetric methods for the determination of aminoglycoside antibiotics.

Method	Aminoglycoside	Matrix	Sample preparation	Type of optical reagent	LOD/LOQ Rec.	Ref.
Spectrophotometric	KAN	Pharmaceutical formulations (suspension)	-	Vanillin (λ = 404 nm)	LOD: 1.24 µg/mL Rec. 100.13%	[82]
	KAN	Pharmaceutical formulations (suspension)	-	Eosin (λ = 548 nm)	LOD: 0.215 µg/mL Rec. 100.34%	[82]
	NEO	Pharmaceutical formulation (tablets)	-	Ninhydrin (λ = 574 nm)	LOD: 3.33 µg/mL Rec. 99.2 – 108.9%	[83]
	AMI	Pharmaceutical formulations (injections),	-	Chloranillic acid (λ = 524 nm)	LOD: 6.49 µg/mL Rec. 94.44 –	[84]

		standard solution			106.4%	
	KAN	Pharmaceutical formulations (injections), standard solution	-	Ascorbic acid (λ = 390 and 530 nm)	LOD: 8.58 – 9.6 µg/mL Rec. 99.98 – 100.09%	[85]
	STR	Water, serum, milk	-	Colorimetry with AgNP-gallic acid probe (ratio between peak intensity at λ_1 = 560 nm and λ_2 = 400 nm)	LOD: 0.02 – 0.1 ng/mL	[77]
Spectrophotometric/ spectrofluorimetric	SPC	Pharmaceutical formulations (vials), human plasma and urine	Plasma: Protein precipitation (ACN)	Benzofuran (colorimetry: λ = 410 nm; fluorimetry: λ_{em} = 530 nm; λ_{ex} = 410 nm)	LOD: Colorimetry: 55 ng/mL Fluorimetry: 4.15 ng/mL	[86]

					Rec. Colorimetry: 97.11% Fluorimetry: 101.19%	
Spectrofluorimetric	NEO, TOB, KAN	Pharmaceutical formulations (tablets, ointments, drops, syrup)	Tablets: grinding and dissolving Ointments: LLE (chloroform)	Acetyloacetone and formaldehyde – Hantzsch condensation (λ_{em} = 471 nm; λ_{ex} = 410 nm)	LOD: 1.6 – 4.93 ng/mL Rec. 99.35 – 100.3%	[87]
	AMI, TOB, NEO, GEN, KAN, STR	Pharmaceutical formulations (tablets, ointments, drops, ampoule, vial, syrup), human plasma	LLE (chloroform) Human plasma: protein precipitation (ACN)	Safaranin (λ_{em} = 545 – 570 nm; λ_{ex} = 519 – 524 nm)	LOD: 1.2 – 1.5 pg/mL Rec. 99.2 – 101.0%	[88]

NEO, TOB, AMI, KAN	Pharmaceutical formulations (injection tablets), human serum, human urine	Plasma: Acidic protein precipitation (TCA) Urine: protein precipitation (MeOH)	2-hydroxyl-1-naphthaldehyde (λ_{em} = 434 nm; λ_{ex} = 366 nm)	LOD: 10 ng/mL Rec. 99.67 – 100.26%	[89]
APR	Pharmaceutical formulations (powder), milk	Protein precipitation (ACN)	Inherent native fluorescence (λ_{em} = 388 nm; λ_{ex} = 335 nm)	LOD: 50 ng/mL Rec. 98.03 – 100.7 %	[76]
APR	Pharmaceutical formulations (powder), milk	Protein precipitation (ACN)	Micelle-enhanced method (enhancing the native fluorescence intensity using sodium dodecyl sulfate)	LOD: 20 ng/mL Rec. 98.10 – 101.40%	[76]

			(λ_{em} = 398 nm; λ_{ex} = 360 nm)		
AMI	Human urine, river water	-	Molecularly imprinted polymer on fluorescent graphitic carbon nitride quantum dots (λ_{em} = 520 nm; λ_{ex} = 374 nm)	LOD: 1.2 – 3 ng/mL Rec. 97.13 – 101.3 %	[80]

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Electrochemical methods

Aminoglycoside antibiotics can also be determined using a variety of electrochemical methods. While the reports on the use of more traditional techniques such as potentiometry or different flavours of voltammetry for AGs determination are rather scarce, a rising trend can be observed in employing these and other electrochemical sensing techniques in the construction of aptamer-based AGs biosensors (aptasensors).

Potentiometric determination of gentamycin and kanamycin was demonstrated employing an ion-selective electrode (ISE) constructed using plasticized membranes containing ionophores based on ion pairs of both aminoglycosides with tetraphenylborate and Acid Chrome Black Special (ABS) [90]. LODs for gentamycin and kanamycin were in the range of 0.5 µg/mL and selectivity constants (gentamycin/kanamycin) were close to unity. Such high values of selectivity constants mean that the electrodes are completely nonselective which limits their usage either to pharmaceutical formulations containing single AGs or to the measurement of total gentamycin/kanamycin concentrations. A voltammetric sensor containing reduced graphene oxide/graphene oxide hybrid□modified electrode was used for the electrochemical detection of tobramycin [91]. The linear response was observed in two concentration ranges: 3.2 ÷ 23.4 µg/mL and 23.4 ÷ 420.3 µg/mL. The LOD value was estimated at 0.9 µg/mL and the sensor was successfully used for determination of tobramycin in human saliva.

Electrochemical aptasensors are gaining more and more interest from the researchers working on easy, selective, quick and reagentless methods for AGs determination, more in line with the stipulations of Green Analytical Chemistry than the more established methods. Briefly, such sensors consist of an aptamer specific for the antibiotic of interest, bound to the surface of the electrode. In the absence of antibiotic molecules, such sensor is in the ‘off’ state, meaning that aptamer molecule/chain has some specific conformation and the sensor, as a whole, has certain electrochemical properties. Introduction of the antibiotic molecules results in their binding with aptamers leading to changes in their conformation and measurable changes in electrochemical properties of the sensor as a whole. Numerous reports describing such aptasensors have been published in recent years. The body of literature on this topic is too extensive for inclusion in this work, and so the reader interested in this topic is advised to read one of the excellent reviews available, such as the comprehensive paper by Mehlhorn *et al.* [92].

Qualitative analysis

Quantitative methods involve aminoglycosides identification, confirmation of structures as well as understanding the mechanisms of interactions, or transformations. The structural configuration of AGs and characterization of interactions between AGs and RNA can be obtained using nuclear magnetic resonance spectroscopy (NMR), in particular ^{15}N-NMR, H-NMR and ^{13}C-NRM [93–96]. Attempts to use X-ray diffraction spectroscopy for AGs characterization have been made as early as the 1960s and 1970s. Due to their amorphous structure and problems with producing diffraction-quality crystals, it can be realized only for selected AGs (e.g. fortimicin) [97]. At present, X-ray crystallography is used to define types of interactions and structures of crystal complexes of AGs with enzymes and RNA [98].

Several studies on structural analysis of AGs using mass spectrometry have also been published. Mass spectrometry was applied to obtain information about interactions between aminoglycosides and other substances (RNA and enzymes) [99,100], to investigate bacterial resistance [101] and to study the structure after chemical modifications [102]. NMR, X-ray diffraction spectroscopy and mass spectrometry provide important support for drug design and bacterial resistance mechanisms investigations.

4. Conclusions

Aminoglycoside antibiotics are valuable antibacterial drugs employed in many areas of human activity. They are effective against a number of microorganisms but must be used with care due to their low therapeutic indices. Since they are relatively inexpensive, cases of unlawful use of these drugs have been reported. Assuring proper food and drugs quality and prevention of environmental pollution requires analytical tools suitable for food, drugs and environmental samples control. Due to high polarity, polycationic character and lack of chromophores, the determination of aminoglycosides is a challenging task both at the sample preparation and final determination stages. The sample preparation step in the context of aminoglycoside antibiotics determination depends heavily on the nature of the sample being analysed and the final determination technique. Protocols used in the analysis of pharmaceuticals tend to be relatively straightforward and generally consist of dissolution, filtration, defatting and derivatization before the actual measurement. The sample preparation workflows applied for food and environmental samples are usually much more elaborate due to both complicated matrices and low levels of analytes concentration. Sample clean-up and analytes preconcentration are conveniently achieved using solid-phase extraction technique. A variety of sorbents can be used for this purpose, from the “traditional” reversed-phase (e.g.

C18 or C8) sorbents through hydrophilic-lipophilic balance, ion exchange, to molecularly imprinted and magnetically active materials. While the selection of the particular sorbent type is determined by the nature of analyte(s) and the final determination technique, molecularly imprinted polymer-based materials seem to be the most promising due to their high selectivity against aminoglycoside antibiotics.

Final determination step may be accomplished using a variety of techniques. In this context, liquid chromatography seems to be the most powerful tool due to the variety of separation modes available and compatibility with several types of selective and sensitive detectors, as evidenced by the body of literature on this topic. Non-separative techniques are well suited for the determination of a single compound. Recent years have seen developments in techniques such as bioassays, quantum dot-based colourimetric applications and aptasensors. They show great potential in the development of low-cost, user-friendly point-of-need tests which could greatly increase the access to AGs analysis. The demand for such solutions might come from consumers who are increasingly aware of the dangers associated with the ubiquitous presence of antibiotics in food and the environment. While the biosensors for such prospective on-site screening tools show overall good specificity, they can fall short in this regard compared to the more conventional methods when analysing samples containing multiple AGs. An interesting development could be the development of an array of different biosensors, e.g. electrochemical aptasensors, akin to the holistic approaches used in electronic noses and tongues, thus leveraging their partial selectivity using multivariate statistical analysis and machine learning models.

Liquid chromatography coupled to a variety of detectors will likely remain the mainstay of qualitative and quantitative determination of AGs, especially in complex matrices and samples containing multiple antibiotics, and the avenues of research in this area are far from exhausted. However, in the view of the recent trends in which an emphasis is placed on the development of green and equitable (more ubiquitous and affordable) analytical techniques, the coming years will likely see exciting developments in the application of microfluidic devices and biosensors.

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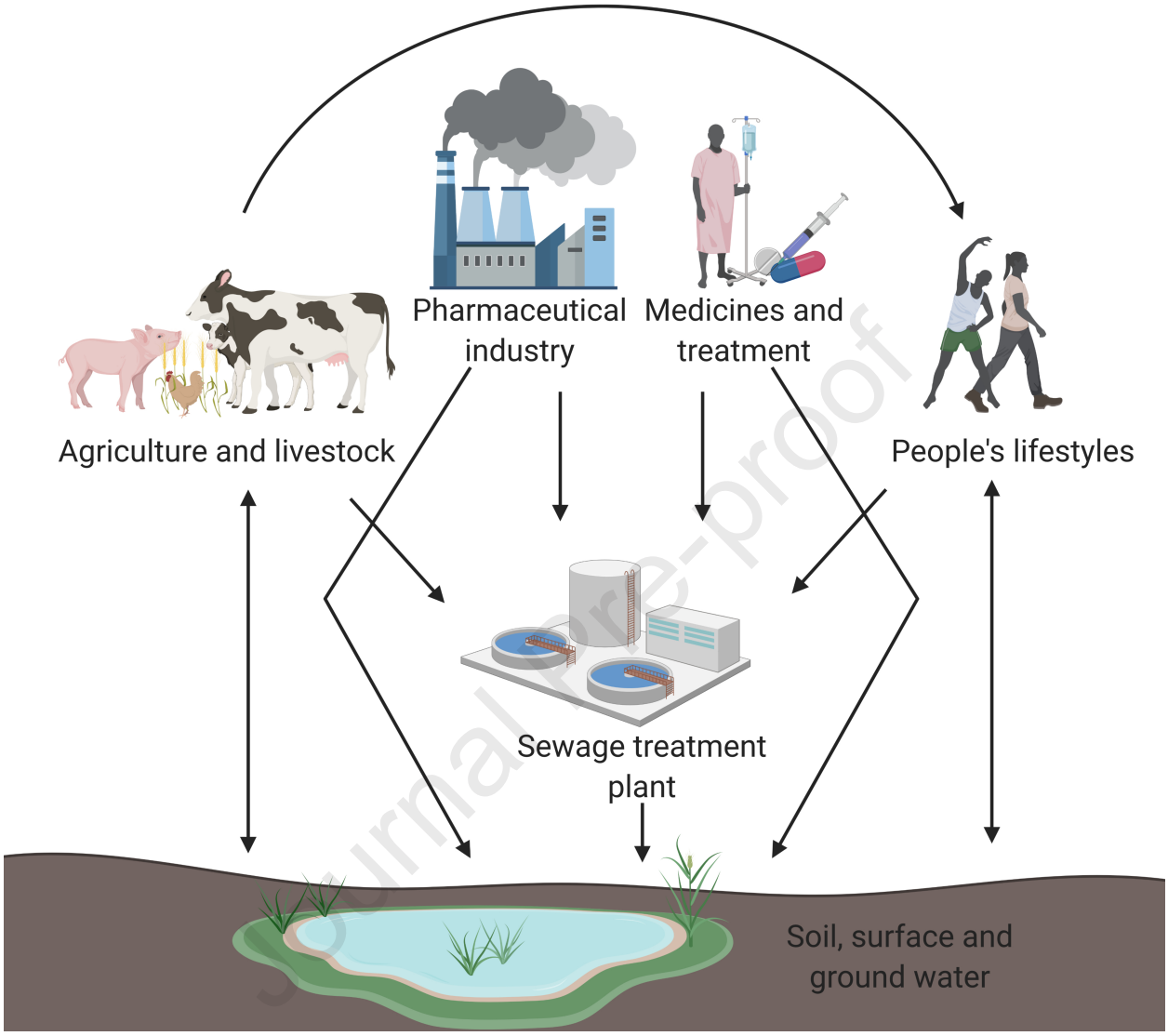
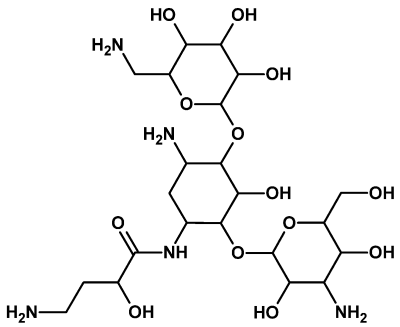
Figure captions

- Fig. 1. Mobility of aminoglycoside antibiotics in the environment. Created with BioRender.com.
- Fig. 2. Structures of selected aminoglycoside antibiotics.
- Fig. 3. Generalised scheme of sample treatment for subsequent determination of aminoglycosides.
- Fig. 4. Example of a chromatogram after derivatization of AGs mixture with FMOC; APR – apramycin, PAR – paromomycin, AMI – amikacin, KAN – kanamycin, TOB – tobramycin, NEO – neomycin, GEN – gentamycin.
- Fig. 5. Comparison of chromatographic separation of 11 AGs under HILIC conditions using SeQuant ZIC-HILIC (A) and Ascentis Si (B) LC columns; AMI – amikacin, APR – apramycin, DHSTR – dihydrostreptomycin, GEN – gentamycin, KAN – kanamycin, NEO – neomycin, PAR – paromomycin, STR – streptomycin, TOB – tobramycin.
- Fig. 6. Determination of kanamycin in vaccine samples based on its effect on the thioglycolic acid-CdTe quantum dots photoluminescence [79]. Aliquots of reconstituted vaccine samples (a) were loaded into a SPE cartridge coupled with a syringe filter and packed with kanamycin-MIP (b). After washing and elution with acidic water, the aliquots were added to a TGA-CdTe quantum dots dispersion probe (c) and the subsequent photoluminescence measurements were carried out using a luminescence spectrophotometer (d). Created with BioRender.com.



AMIKACIN



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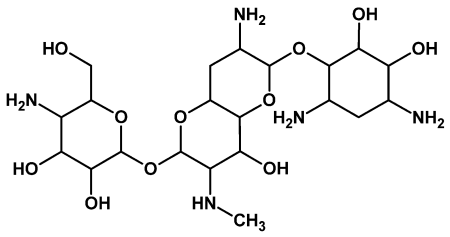

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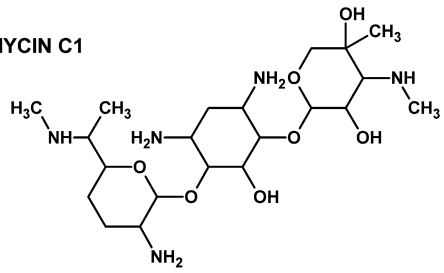

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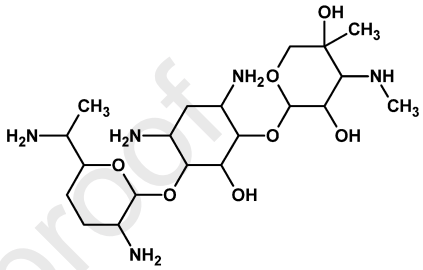

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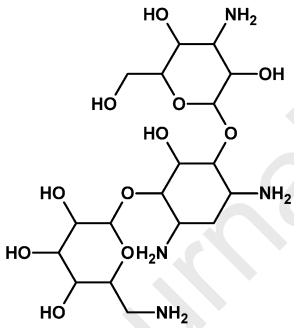
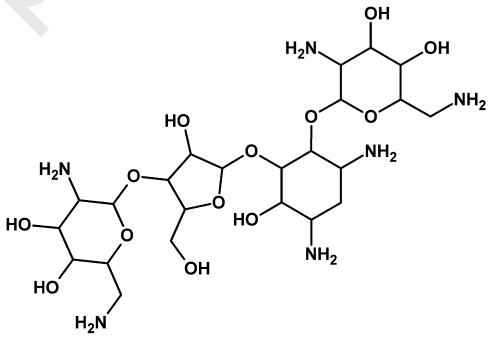
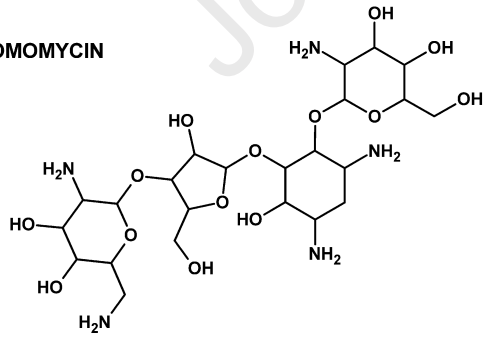


NEOMYCIN B



PAROMOMYCIN



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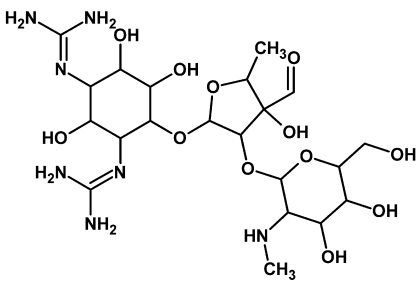

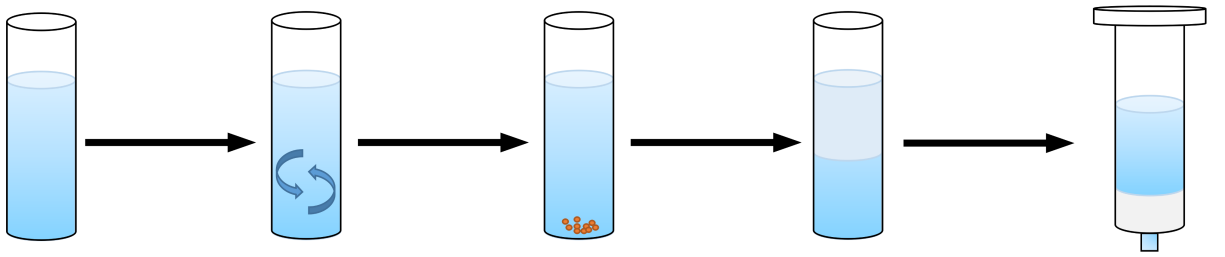

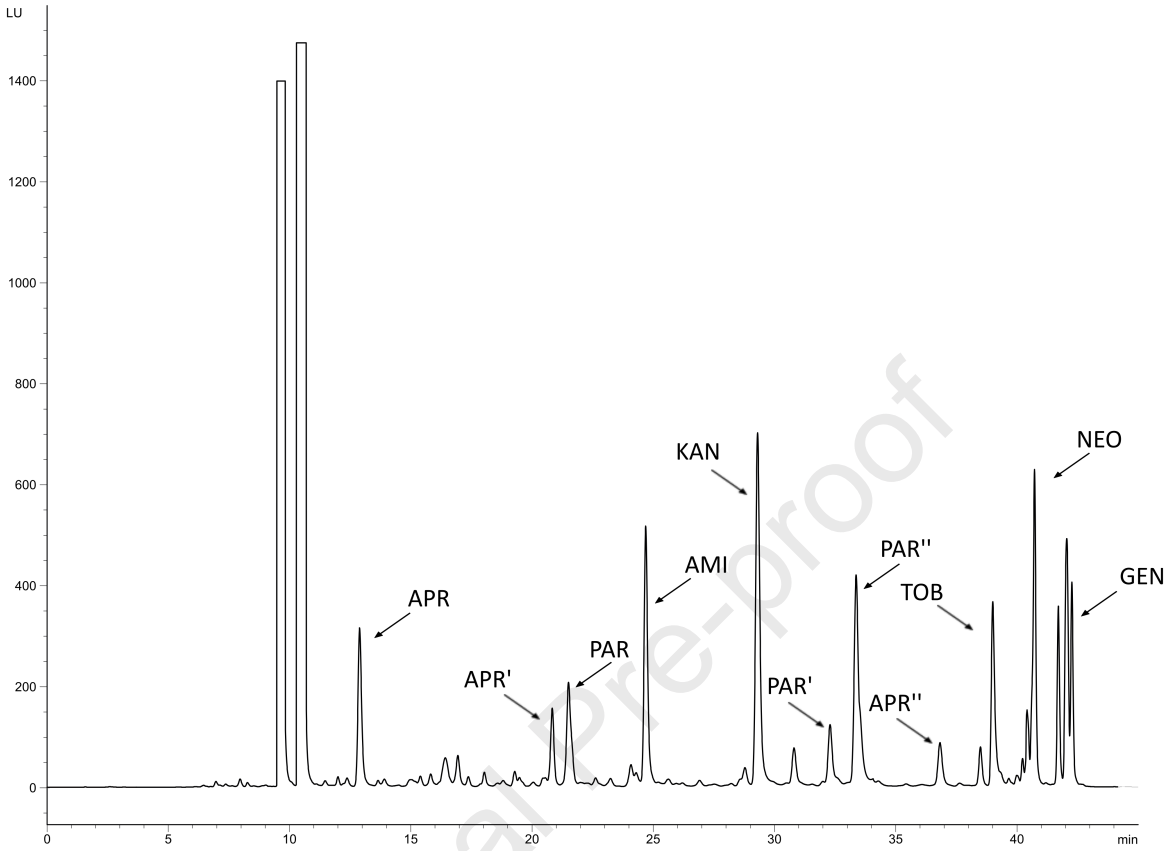

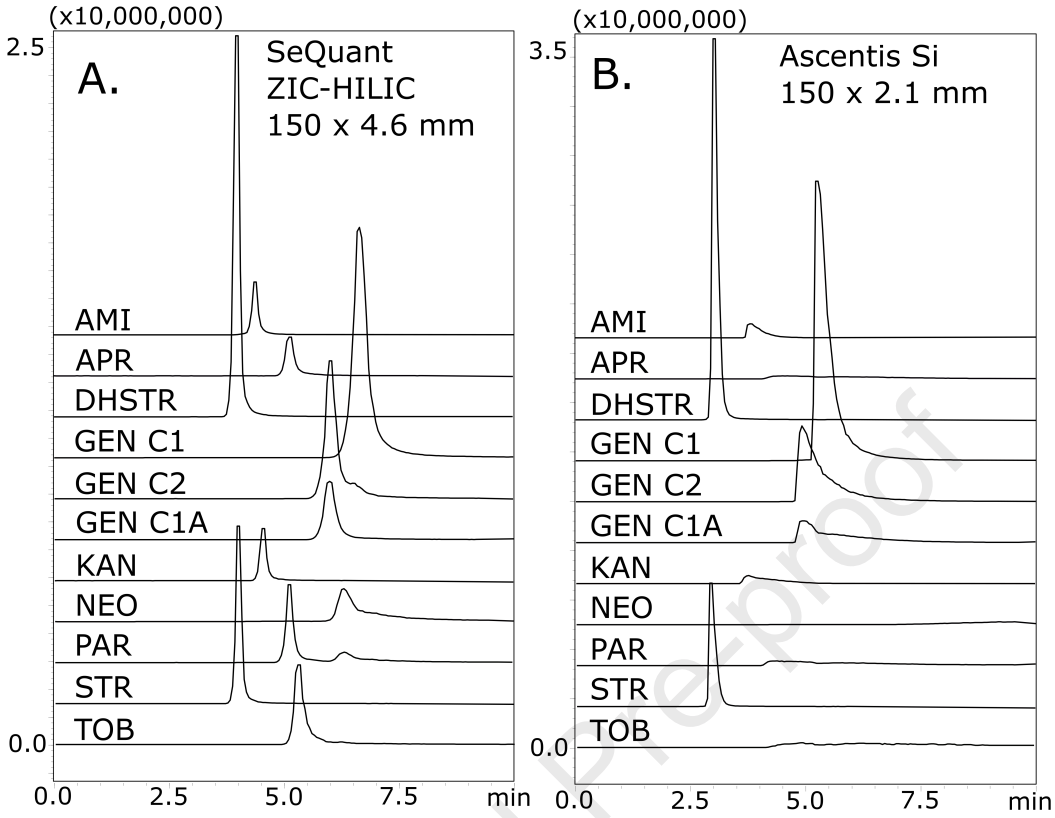

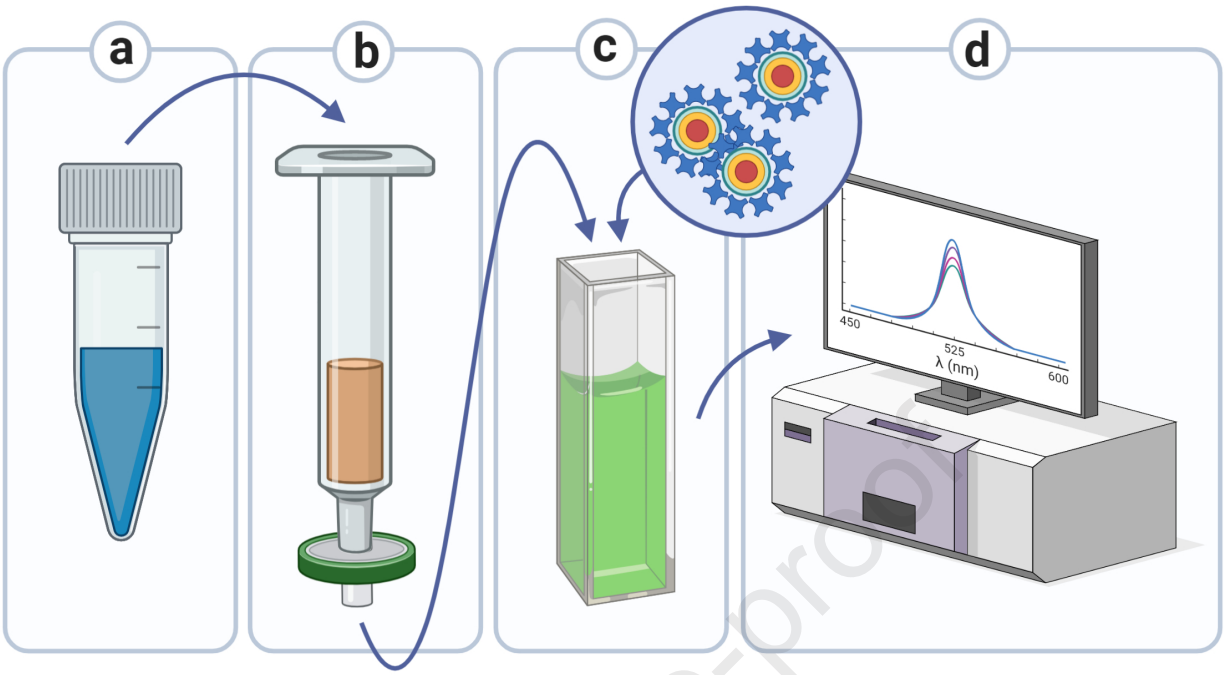

- Reliable analytical methods are needed for determining aminoglycoside antibiotics.
- We review the state-of-the-art in sample preparation and detection techniques.
- Trends in the development of both LC-based and emerging methods are discussed.

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: