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Abstract

Aminoglycosides are mostly used as veterinary antibiotics. In France, their consumption accounts for about 10% of all prescribed animal medicine. Due to their high polarity nature (log Kow < -3), they require chromatographic separation by hydrophilic interaction liquid chromatography or ion-pairing chromatography. This study presents the development of an ion pairing liquid chromatography with alkanesulfonates coupled to tandem mass spectrometry for the analysis of 10 aminoglycosides (spectinomycin, streptomycin, dihydrostreptomycin, kanamycin, apramycin, gentamicin, neomycin and sisomicin) in wastewater samples. The novelty of this method lies in the addition of the ion paring salt directly and only into the sample vial and not in the mobile phase, lowering the amount of salt added and consequently reducing signal inhibition. The optimized method was validated and showed satisfactory resolution, performances suitable with the analysis of aminoglycosides in wastewater samples, with limits of quantifications less than 10 ng/mL for most of the compounds, low matrix effects, high

accuracy (85%-115% recoveries) and reproducibility (2%-12%RSD). It was then applied successfully to raw and treated wastewater samples.

Keywords: Aminoglycosides, wastewater, ion-pairing chromatography

Highlights:

- In vial addition of salt, limiting the presence of salts in the LC-MS/MS system
- A sample preparation reduced to a simple addition of salt in the vial
- Low matrix effect allowing external calibration with solvent standards

1. Introduction

During the past decade, aminoglycosides (AGs) became one of the most widely used veterinary antibiotics in both bovine and pork herds [1–3], because of their wide action range for both Gram-negative and Gram-positive bacteriaIn France, while the global antibiotic consumption is decreasing constantly (divided by 3 in 12 years), aminoglycosides portion is increasing with up to 10% prescription share in 2017 [2,3]. Furthermore, aminoglycosides are poorly absorbed due to their high polarity nature and are excreted unchanged in urine [4,5]. Aminoglycosides antibiotics are also used in human medicine formulation [6,7], increasing the probability of their presence in wastewaters.

Despite their increasing consumption, analytical procedures for environmental matrices are very few and aminoglycosides are still among the least analysed antibiotics today. The main reason is probably their highly polar nature, with log Kow comprised between -3 for spectinomycin and -9 for neomycin. Indeed, as noted by Reemtsma et al. [8], this kind of very polar molecules requires specific analytical tools which do not currently exist and which need to be developed. Analysis of AGs are more frequent in food-related samples as reported by Glinka et al. [9] such as meat [10–12], milk [12,13] or honey [14–18]. Maximum residues limits are defined by food regulations and analytical method performances are set to respect these values. In the environmental field, there is no regulation or monitoring regarding aminoglycosides. To the best of our knowledge, only a few studies deal with aminoglycosides analysis [19–22].

Whatever the matrix of interest, separation methods for AGs analysis are based on two major techniques: Hydrophilic Interaction Liquid Chromatography (HILIC) [4,5,11,23] or Ion Pairing Liquid Chromatography (IPLC) [17,24], often followed by tandem mass spectrometry detection. When HILIC is used, a wide variety of column chemistry can be employed for the analysis of aminoglycosides..

Alechaga et al. obtained poor peak shapes using bare silica [25], Ianni et al.[26]used a two-dimensional LC with HILIC in 1st, but required between 51 and 70 minutes to obtain selectivity.Guillarme tested a variety of HILIC columns and only obtained resolution with zwiterrionic ones[27]. Altogether, HILIC separations lacks of robustness, as variations in mobile phase composition, pH, buffer concentration or temperature can have a very noticeable effect on selectivity and retention of compounds [28].

In case of very polar compounds as aminoglycosides, Mokh et al. [20] reported that ion-pairing liquid chromatography could represent a more suitable and powerful technique, with better retention time consistency. One of the first and most critical steps of IPLC optimization is the selection of the counter ion. In most reported works in IPLC, heptafluorobutyric acid (HFBA) [19,29–31], perfluoropentanoic acid (PFPeA) [32], trichloroacetic acid (TCA) or trifluoroacetic acid (TFA) [29] were used, added in the mobile phase. To match chromatographic conditions, ion-pairing reagents were also added in the sample vial. Nevertheless, those additives are known to cause signal inhibition in mass spectrometry [33], increasing detection and quantification limits. A comparison of HILIC and IPLC with HFBA as counter ion was realised by Gremilogianni et al. [30] and concluded to greater performances of the HILIC method, because of high ion suppression caused by IPLC. Moreover, introduction of high salt content mobile phases in the mass spectrometer source also leads to more frequent instrument maintenance. To circumvent this problem, Lehotay et al. [34] proposed an IPLC method for the analysis of drug residues, adding ion-pairing reagents only into the injection vial, thus reducing the amount injected in the LC column and in the mass spectrometer, preventing some down-time of the instrument for cleaning and maintenance. The ion suppression caused by the IP reagent was consequently reduced, allowing to achieve better quantifications limits. Wang et al. and Amelin et al. [31,35] also proposed this “in vial only addition” method, using HFBA as ion pair whereas Lehotay used sodium heptanesulfonate, known as more volatile reagent, in

84 order to further reduce signal suppression. However, in all studies with only in vial addition,
85 little to no resolution was obtained, with AG separation spread only over 0.5 to 1 min, which
86 may cause analytical difficulties in complex and charged matrices.
87 Based on these observations , the objective of this work was to develop and optimize an “only
88 in vial addition” IPLC-MS/MS method with satisfactory resolution of 10 AGs and to evaluate
89 its suitability for their surveillance in environmental waters. First, various alkanesulfonate
90 counter-ions with different carbon chain length were tested to evaluate retention and separation.
91 Chromatographic conditions such as organic mobile phase, gradient or isocratic elution,
92 mixture of IP reagents were developed to obtain good separation, and minimize matrix effects.
93 The final method was validated and applied on water samples from both wastewater treatment
94 plants around Lyon and from the Rhône river (France). This is then the first method reporting
95 resolution of aminoglycosides with only “In vial addition” IPLC.
96
97 2. Experimental
98 2.1. Chemicals and reagents
99 Apramycin (APR), dihydrostreptomycin (DHSTREP), gentamicin (GEN), kanamycin (KAN),
100 neomycin (NEO), spectinomycin (SPEC), streptomycin (STREP) and sisomicin (SISO) were
101 purchased from Sigma Aldrich (Saint Quentin Fallavier, France) in VETRANAL quality or
102 equivalent purity (>98%). The GEN standard contained three distinct molecules: Gentamicin
103 C1 (GEN C1); Gentamicin C1A (GEN C1A) and Gentamicin C2 (GEN C2) (Figure S1). Stock
104 solutions (1 $mg.mL^{-1}$) of each aminoglycoside were prepared by dissolving about 10 mg
105 powder, accurately weighted in 10 mL of water/methanol (1/1, v/v) in high density polyethylene
106 Wheaton vials were stored at -18°C.Accurate concentration was then calculated taking each
107 standard purity in account . Autosampler vials and centrifuge tubes in polypropylene (PP) were

used to prevent adsorption of the analytes on glass. Calibration solutions of each analyte (500 ng.mL^{-1}) were prepared by diluting individual stock solutions in water/methanol (1/1, v/v). Alkanesulfonate salts ranging from butanesulfonate to decanesulfonate (Figure S1) were acquired from Tokyo Chemical Industry, Belgium to be used as ion-pairing salts. Individual salt solutions were realised at 75 mM in water and stored in the fridge at 4°C during one month. Water (LC-MS grade) was obtained from Fisher Scientific (Illkirch, France), methanol (MeOH) and acetonitrile (ACN) (LC-MS grade) from Honeywell (Seelze, Germany) and formic acid (UPLC-MS grade) from Biosolve (Dieuze, France).

2.2. Real sample collection and preparation

Three kinds of water matrices were collected: river water for the optimization and raw and treated wastewaters (WW) for application of the optimized method. River water was grab sampled in the Rhône river (France). WW were collected at 3 different WW treatment plants in the region of Lyon (France).

Samples were stored at -20°C before analysis. With the optimized method, after thawing, a 2 mL aliquot was sampled and transferred to a PP centrifuge tube, centrifuged at 10000 rpm for 5 min (3K3OH, Sigma, Germany). Then 200 µL of supernatant was transferred into a 1 mL PP vial together with 200 µL of sodium hexanesulfonate solution and 200 µL of sodium heptanesulfonate solution. The vial was then capped and agitated on a rotor mixer for 20 seconds.

2.3. Ion Pair Liquid chromatography coupled to tandem mass spectrometry (IPLC-MS/MS)

The system used was an Agilent (Massy, France) 1200 Series High-Performance Liquid Chromatography system with a binary pump. The column was a Kinetex XB-C18, 100*2.1mm, 1.7 µm from Phenomenex (Le Pecq, France). Optimized IPLC conditions were as followed: a

binary mobile phase was used with a flow-rate set to 300 µL.min^{-1} for a total run time of 15 min, with the column maintained at 40°C. Mobile phase A was an aqueous solution of 0.1% formic acid, and B was a mixture of ACN/MeOH (1/1) with 0.1% formic acid. The separation was performed with an isocratic mobile phase at 10% B for 7 min. B was then increased to 90% for 5 min and then decreased back to 10% for 3 min starting re-equilibration of the column. An equilibration time of 5 min (i.e. 6 column volumes) was realised before each injection, leading to a total run time of 20 min. The sample injection volume was 10 µL. The final injection solvent was composed of 90/10 H_2O/(MeOH/ACN) containing 50 mM of both hexanesulfonate and heptanesulfonate sodium salts.

A 5500 QTrap from Sciex® (Les Ulis, Fance) was used in Multiple Reaction Monitoring (MRM) mode with positive electrospray ionization. Source parameters are detailed in Table S1. MS/MS detection was optimized by infusion of individual standard solutions at 100 ng.mL^{-1} via syringe pump at a flow of 10 µL.min^{-1} and are presented in Table S2. For SPEC, both protonated and water adducts showed a similar sensitivity; we chose to monitor both ions, and use the H_2O ion for quantification and the $[M+H]^+$ adduct for confirmation [36]. NEO and SISO also formed a doubly charged ion in the ionisation source, it was decided to follow both ions.

2.4. Method validation

Limits of quantification (LOQs) were evaluated as the concentrations leading to a signal-to-noise ratio of 10. The method linearity for each molecule was determined by injection of standards mixtures from 0.5*LOQ to 50*LOQ. Intra day repeatability and intermediate precision were both evaluated during three days. Each day, a calibration curve for each component was freshly prepared and injected, followed by three standard solutions spiked at three concentration levels: LOQ, 2*LOQ and 10*LOQ, also freshly prepared. For each analyte and each level, the concentration was computed with the calibration curve and the accuracy

158 calculated with the mean of the 3 replicates versus the nominal concentration. Intraday
159 repeatability was determined by calculating the relative standard deviation (RSD) of replicates
160 injected the 2nd day and interday precision was determined by calculating the RSD on the three
161 days means.
162 Matrix effects were evaluated for each analyte by comparing a calibration curve prepared in
163 pure LC-MS grade water and in river water, after centrifugation. The ratio of the slopes was
164 considered as matrix effect indicator.
165
166 3. Results and discussion
167 3.1. Adsorption of GEN on container material
168 During the first experiments, decreases appeared on several signals, especially for GEN. A
169 comparison between glass and PP vial was carried out to test for adsorption of the three distinct
170 molecules composing GEN. Figure 1 illustrates the significant signal diminution, of about 50%
171 for each component of the GEN mix when using glass vials. Moreover, the variations in-
172 between vials were also increased in glass container. PP vials were hence used for all the study.
173 No adsorption difference was noted between the three gentamicin components as their
174 distribution was unchanged between glass and PP vials: 29% GEN C1; 34% GEN C1A and
175 37% GEN C2.
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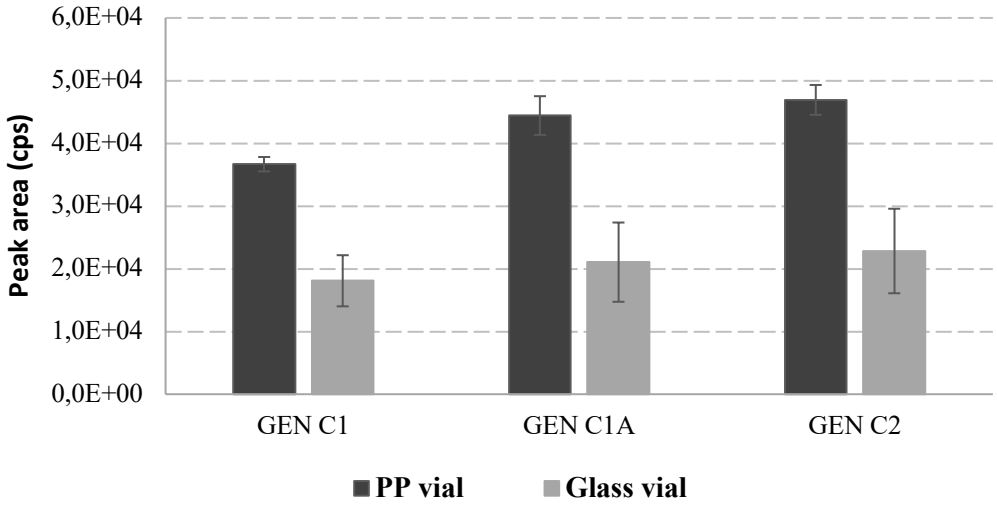
Figure 1: Comparison of the LC-MS/MS signals of GEN C1, GEN C1A and GEN C2 solutions at 200 ng.ml^{-1}, left for 24h at 4°C in PP or glass vials (n = 3).



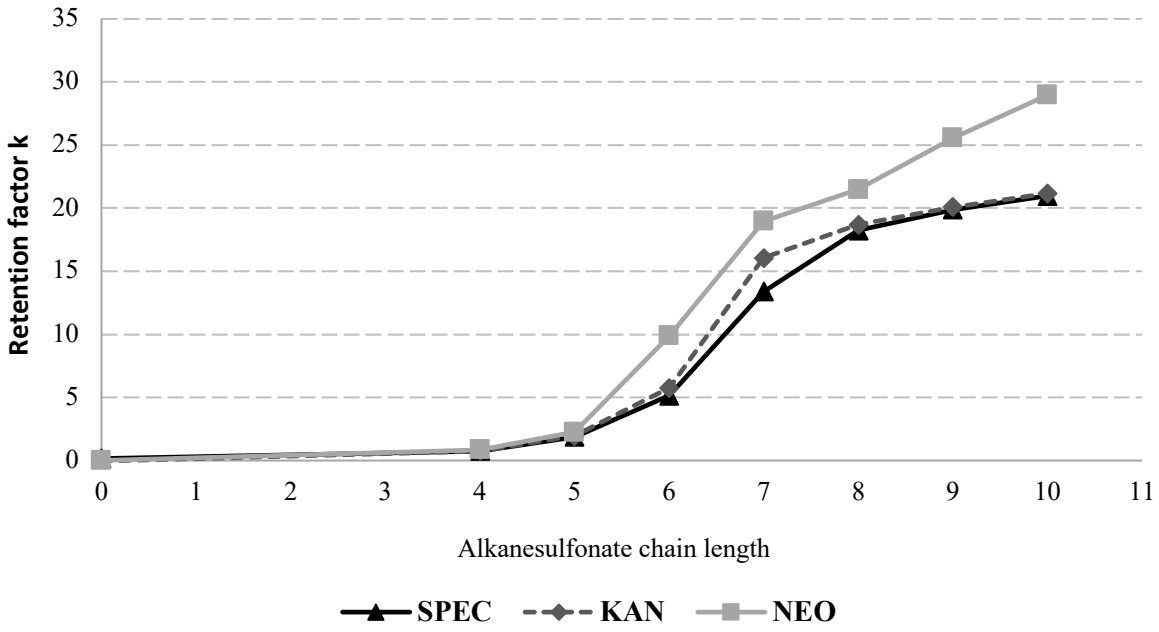
3.2. IPLC-MS/MS method optimization

3.2.1. IP salts choice

Alkanesulfonates ion-pairing salts from sodium butanesulfonate to sodium decanesulfonate were tested for the separation of AGs. Each salt was evaluated individually at 50 mM, by mixing 300 µL of standard solution with 600 µL of IP salt at 75 mM in the injection vial, except for nonanesulfonate and decanesulfonate, tested at 25 mM due to their lower critical micellar concentration (estimated from [37] at 65 mM and 32 mM for nonanesulfonate and decanesulfonate, respectively). An injection was also realised without addition of IP salt. The separation was evaluated by considering the retention factor (k). An illustration of the results for three aminoglycosides with dispersed retention times (SPEC, low; KAN, middle, NEO, high) are presented in Figure 2. It can be observed that when no salt was added, all compounds were eluted in the column dead volume, without separation. On the other hand, a separation was possible in the presence of salts, with k increase with the carbon chain length. Same behaviour was observed for all targeted AGs. Satisfactory separation was obtained when using

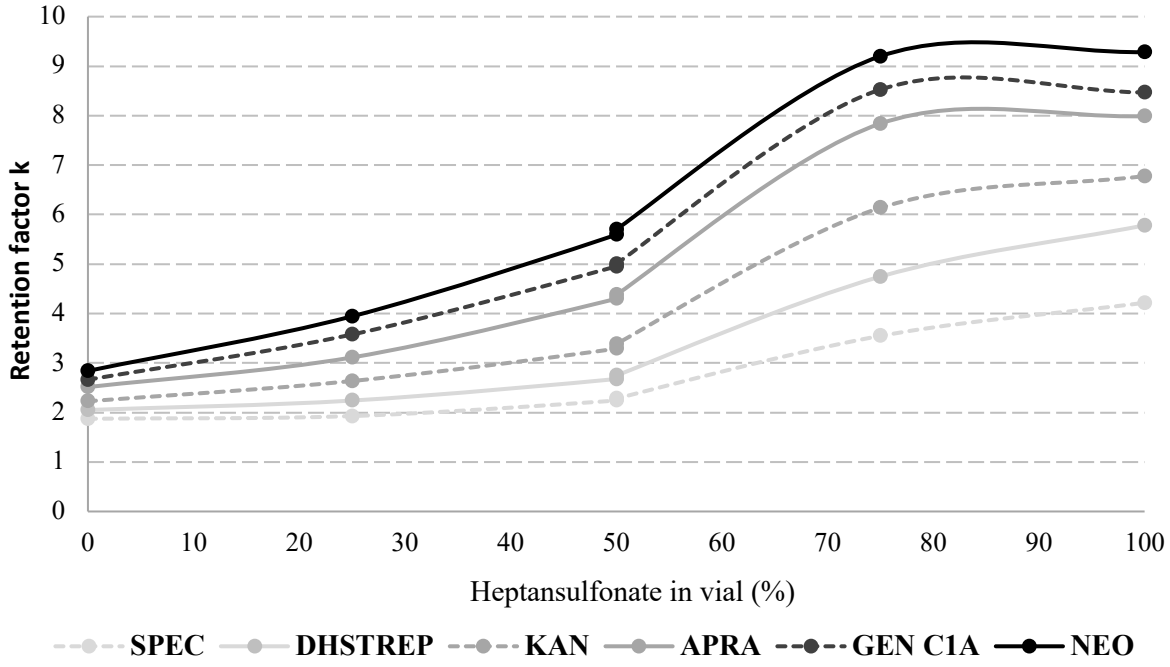
ion pairing alkanesulfonate salt between n=6 and n=7. For n>7, similar retention was observed for compounds that were previously separated with heptanesulfonate salt.

Figure 2: Evolution of retention factor k for the aminoglycosides SPEC, KAN and NEO as a function of the ion pair carbon chain length



To further optimize the separation, different proportions of hexanesulfonate (IP6) and heptanesulfonate (IP7) salts were tested: 0/100, 25/75, 50/50, 75/25 and 100/0. For all AGs, an increase of the IP7 percentage resulted in an increase of the retention factor (Figure 3). The 50/50 proportion of hexanesulfonate and heptanesulfonate ion pairing salt was finally chosen, which allowed an satisfactory separation in 8 minutes . If more retention or faster separation is required, the method could be easily modulated by modifying IP ratios.

Figure 3 : Evolution of retention factors k with proportion of heptanesulfonate in vial



3.2.2. Organic solvent mobile phase

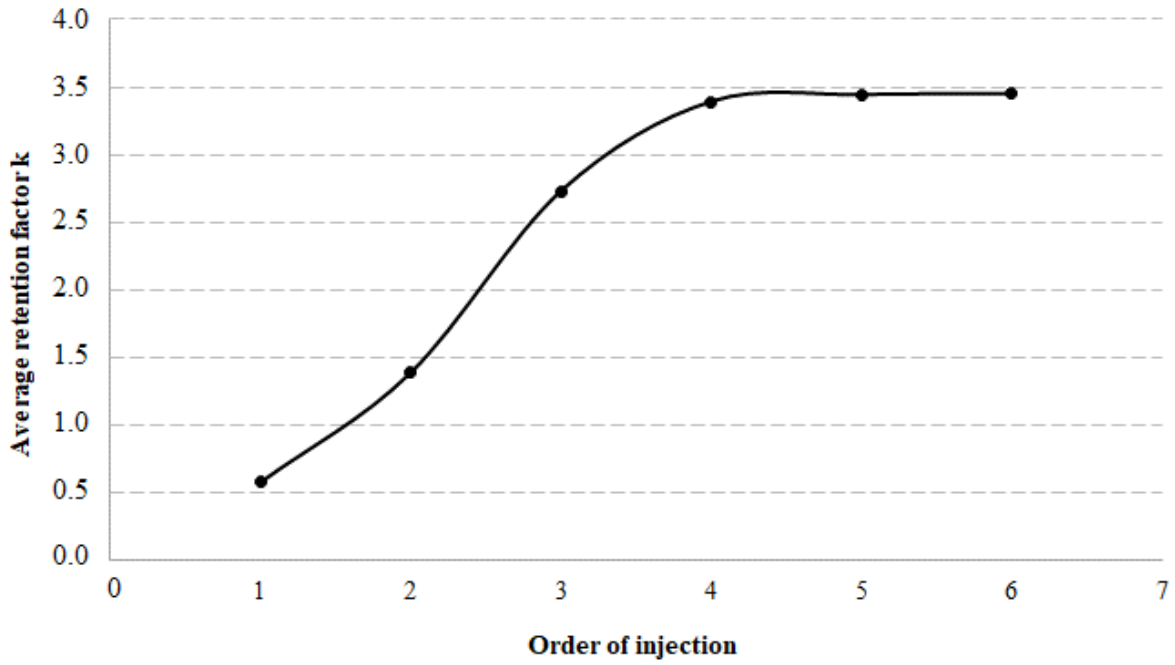
After the selection of the ion pairing salts, interest was moved to the organic solvent used (ACN or MeOH) to both reduce the analysis run time and improve separation. When ACN was used in the mobile phase, all compounds eluted earlier, reducing the analytical time, but also reducing the resolution between compounds that elutes at close retention times i.e. streptomycin and dihydrostreptomycin or the gentamicin components. A one to one mixture of MeOH/ACN was finally selected to obtain a satisfactory separation of critical pairs while keeping a shorter analysis time than using only MeOH. The presence of MeOH also allowed better ionisation of each molecule in the MS source (data not shown).

3.2.3. Column conditioning and storage

To ensure reproducibility of analysis, the column needed to be conditioned with IP salts before each sequence. To determine the number of injections needed to obtain a repeatable retention

times, a serie of six standards injections in IP6/IP7 (50/50, v/v) salt was carried out on a clean column (with salt removed, with the procedure described thereafter). The evolution of the retention factors with the number of injections is presented in Figure 4 and it can be noticed that constant retention factors were obtained after four injections of IP salts. Therefore, in order to maintain repeatable retention times, six consecutive injections of IP6/IP7 (50/50, v/v) salts were realized prior to any sequence of analysis. The final chromatogram is presented in supplementary materials (Figure S2).

Figure 4 : Evolution of the average retention factor of all targeted AGs with injection number



One of the major drawbacks in using ion pairing chromatography is the possible clogging of the LC column. Removal of the salts after an analysis sequence is important to preserve the column and avoid increasing pressure, or even column blockage. The cleaning procedure proposed included three steps. First the LC was disconnected from the mass spectrometer and the flow was directed to a waste bottle. A 75/25 H_2O/MeOH mixture was then flowed through

the column at 100 µL.min^{-1} for 15-20 min for optimal salts solubilisation. Then, 50/50 H_2O/MeOH was flowed at the same rate for 15 min, resulting in a higher pressure, allowing further penetration in the column particles pores and further salts solubilisation. Finally, 25/75 H_2O/MeOH mixture was flowed at 300 µL.min^{-1} to equilibrate the column for storage and to allow the C18 chains to be reconditioned.

3.3. Method validation

3.3.1. Linearity of the method

First, approximate quantification limits of aminoglycosides were determined by injecting replicates (n=3) of standard solutions , based on signal-to-noise ratios of 10. Aminoglycosides response linearity was then determined from the injection of standards. Each compound displayed a good linearity over the selected range, with determination coefficients (R^2) greater than 0.99 (Table 1).

	Retention time (min) (%RSD[b])	LOQ ($ng.ml^{-1}$)	Linearity		Transition ratio (%RSD[b])	LOQ		2 LOQ		10 LOQ	
			Range ($ng.ml^{1}$) (0.5LQ-50LQ)	R^2		Mean (%RSD[(a/b)])	Accuracy (%)	Mean (%RSD[(a/b)])	Accuracy (%)	Mean (%RSD[(a/b)])	Accuracy (%)
SPEC	3.4 (1.0)	9.6	5-500	0.992	0.58 (1.3)	9.0 (5/33)	94	20.2 (5/4)	105	96.2 (1/6)	101
SPEC+H2O	3.4 (1.0)	9.6	5-500	0.998	0.29 (0.7)	10.2 (5/18)	107	19.7 (4/5)	103	98.2 (4/2)	103
STREP	4.4 (1.3)	5.2	2-260	0.994	0.54 (2.3)	5.4 (4/20)	104	10.3 (3/2)	99	50.7 (1/4)	98
DHSTREP	4.4 (1.1)	0.7	0.3-35	0.995	0.37 (3.0)	0.7(19/11)	96	1.5 (12/10)	104	7.1 (6/5)	101
KANA	5.2 (1.2)	3.1	1-160	0.991	0.65 (1.9)	3.5 (6/8)	111	6.4 (2/4)	101	31.5 (4/0.6)	100
APRA	5.8 (0.3)	17	8-850	0.996	0.86 (1.6)	15.7 (6/23)	92	35 (6/12)	103	170.9 (2/11)	100
GEN C1	6.5 (0.4)	45	22-2230	0.995	0.84 (3.5)	40.0 (14/4)	90	87.8 (7/10)	98	443.8 (7/2)	100
GEN C1A	6.2 (0.5)	28	14-1420	0.999	0.57 (3.3)	30.4 (4/24)	107	55.5 (4/7)	98	285.2 (2/4)	100
GEN C2	6.3 (0.8)	64	30-3200	0.998	0.60 (2.3)	65.5 (6/10)	103	122.3 (6/4)	96	620.3 (1/6)	98
NEO	6.8 (0.1)	465	230-23234	0.995	0,49 (1.4)	577 (13/3)	124	1022.6 (7/5)	110	4671.5 (4/5)	101
NEO 2+	6.8 (0.1)	464	230-23234	0.994	0.76 (6.2)	/	/	941.3 (12/7)	101	4703.9 (9/2)	101
SISO	6.0 (0.5)	111	55-5531	0.995	0.54 (1.5)	120.4 (11/8)	109	22.3 (11/7)	100	1116.8 (7/3)	101
SISO 2+	6.0 (1.5)	111	55-5531	0.994	0.36 (12.9)	111.7 (17/21)	101	222.1 (8/12)	100	1064.4 (3/7)	96

Table 1: Validation results: Linearity and reproducibility at 3 different levels (a: intraday n=3; b: interday n=3 days)

3.3.2. Limits of quantification

Quantification limits of the optimized analytical method were calculated according to the obtained calibration curves and the coefficients of variation, and were comprised between 0.6 and 460 $ng.mL^{-1}$ (Table 1) therefore consistent with the literature for aqueous environmental matrices (Table 2). Mass spectrometry response factors of NEO and SISO were lower leading to higher LOQs than for the other analytes [20]. It is worthnoting that previous studies included a pre-concentration step (solid phase extraction, lyophilisation) whereas the method optimised in the present work includes a simple addition of salts in the injection vial. It is therefore just as sensitive while being faster and less solvent consuming.

For STREP, DHSTREP and KAN, the method developed in this work resulted in improved LOQ by a factor of 10, 50 and 5, respectively, regarding the work of Mokh et al. [20], who used pentafluoropropionic acid as IP in the mobile phases with a 16 min run time. Qiu et al. [21] reported similar LOQs, after a preconcentration step by a factor of 10.

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Analytes	Matrix	Sample preparation	LC conditons	LOQs (ng.ml^{-1})	Ref.
SPEC, STREP, DHSTREP, GEN, KAN, APRA, SISO, NEO	Wastewaters	SPE	IPLC-MS/MS	15-45	[21]
SPEC, STREP, DHSTREP, GEN, KAN, APRA, SISO,NEO	Wastewaters	Lyophilisation	Mixed mode LC-MS/MS	2-20	[22]
SPEC	Liquid hog manure	SPE	HILIC-MS/MS	6	[23]
SPEC, STREP, DHSTREP, GEN, KAN, APRA, SISO, NEO	Wastewaters	Centrifugation and dilution by salt additions	IPLC-MS/MS	0.3-60 (100-500 for SISO and NEO)	This work

281 **Table 2 : Quantification limits obtained in different environmental matrices**

3.3.3. Intraday repeatability, intermediate precision and recovery

Results of both repeatability and intermediate precision experiments are compiled in Table 1. Concerning the intraday repeatability, RSD were measured inferior to 20% at LOQ level for all compounds, showing good repeatability from one analysis to another. At 10*LOQ level, RSD are further reduced, below 5% for 80% of the followed molecules. For intermediate precision, at LOQ levels, most RSD were measured below 25%, except for SPEC, evaluated at 33%. As $[M+H]^+$ ion was only monitored for confirmation, this higher variation had no impact on the validation process. These variations were deemed acceptable based on the paper from Rambla-Alegra et al. [38], stating that, for analyte concentration around the $ng.g^{-1}$ level, RSD below 30% are reasonable. At levels 2*LOQ and 10*LOQ, all RSD were below 12%. For all levels, quantification was accurately realised with calculated values within ±15% of the nominal concentration. As responses of the MRM transitions corresponding to NEO 2+ were very variable at the LOQ level, it was excluded from the reproducibility results. This exclusion did not impact the results as the singly-charged ion for neomycin was well detected at the LOQ.

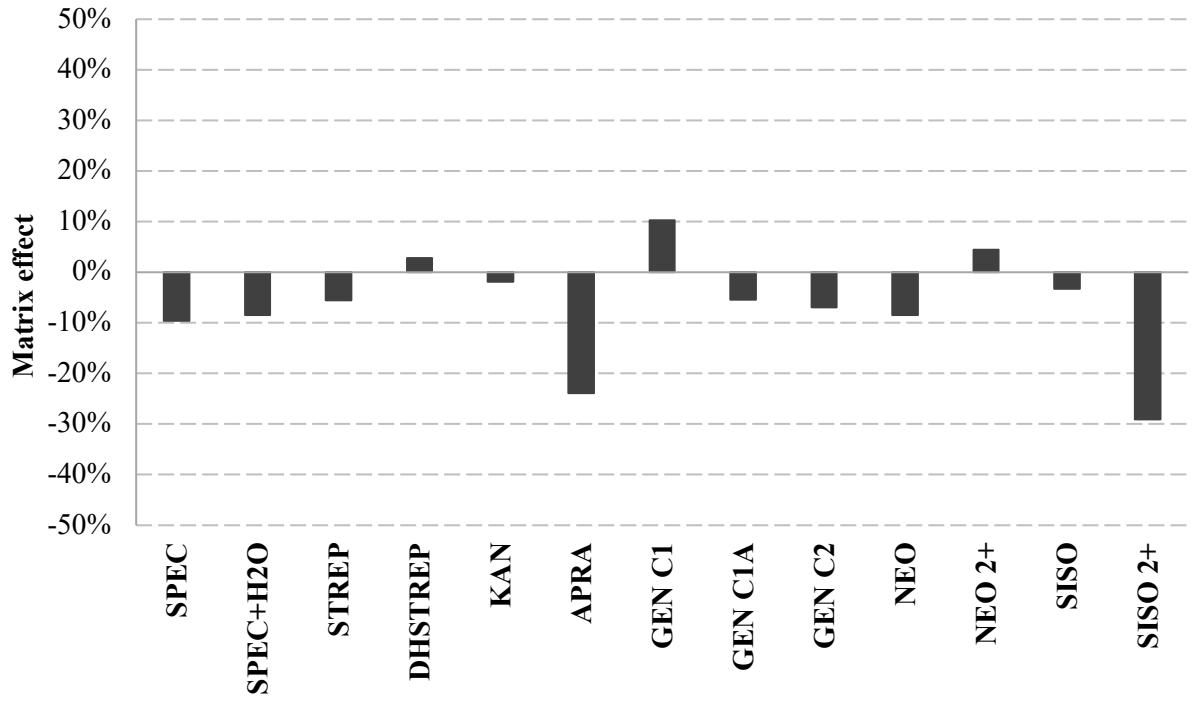
Both variations on the retention time and the ratio between the two followed transitions were also recorded, and presented in Table 1. Throughout the analysis, retention time variations were all lower than 0.1 min, and ratio variations were all below 5%RSD, except for the doubly charged transitions we followed for NEO and SISO. These higher variations could be explained by the tendency of the molecules to form either the single charged ion or the doubly charged, with no predominant form.

Qiu et al. reported recoveries between 66% and 116% of their analytes at 20, 50 and 100 $ng.mL^{-1}$ with inter-day RSD below 16%. The method presented here reduced the recoveries range to 85%-115%. Direct injection of the sample avoids some losses due to lyophilisation step.

3.3.4. Matrix effects

The matrix effects, expressed in %, are presented in Figure 5. For most molecules, matrix effects were comprised within -20% and 20% and was considered negligible. Therefore the quantification could be performed from a calibration realised in solvent. Only APRA and the doubly-charged ion for SISO presented higher matrix effect (a signal inhibition) and would require a matrix match calibration or the use of isotope labeled standards. Further dilution of the sample could reduce those matrix effect to enable only calibration in solvent.

Figure 5 : Evaluation of matrix effects for aminoglycosides in river water (calibration curve : LQ-50*LQ)



3.4. Application to WW samples

Six samples of raw and treated WW were analysed unspiked and spiked at 10*LOQ with all the aminoglycoside standards. Blank controls composed of H_2O/ACN/MeOH (90/5/5) +0.1% formic acid, corresponding to the chromatographic initial conditions, were injected during the

analytical batch, in the same way as the standard solutions and the samples No aminoglycoside was detected in unspiked samples neither raw nor treated wastewaters. Chromatograms are supplied in supplementary materials (Figure S2). In similar studies, Löffler et al.[19] and Mokh et al.[20] only detected GEN (average 4 $ng.mL^{-1}$ and 0.3 $ng.mL^{-1}$, respectively) in hospital effluents, as GEN can also be used in human medicine. Regarding spiked samples, accuracy at 10*LOQ was calculated and was comprised between 75% and 125%, as reported in Table 1. These results demonstrate again the negligible matrix effects induced by this method, even when analysing more complex environmental water samples, such as wastewaters.

4. Conclusion

The analytical method developed in this study allows the simultaneous determination of 10 aminoglycosides in environmental water samples, with simple, fast, and eco-friendly sample preparation. Ion pairing liquid chromatography was developed with a novelty : adding the ion-pairing salt directly in the sample vial instead of the mobile phases reservoirs. This allowed the use of lower quantity of ion-pairing salt per analysis, therefore limiting their negative impact in mass spectrometry such as signal inhibitions, while still obtaining a good and consistant separation. The method was found sensitive and reliable in environmental analysis with almost non existent matrix effects, recoveries comprised between 85% and 115%, with low variations across intra and inter-day analyses. This work is the 1st to demonstrate the ability of “in vial addition only” IPLC-MS/MS methods to monitor accurately, in environmental waters, highly polar molecules and could be adapted to other substances of this type, with modulation of IP reagents ratios or chain length.

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