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Sample preservation for the analysis of antibiotics in water

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Abstract

This paper describes a stability study performed for 56 antibiotics belonging to 9 different groups - macrolides, tetracyclines, fluoroquinolones, quinolones, penicillins, cephalosporines, lincosamides, sulfonamides and nitroimidazole antibiotics - in purified water samples fortified with the selected compounds at 10 ng/ml. For this purpose, three different sample preservation modes were tested with the aim of avoiding biotic and abiotic degradation: i) storage at -20ºC, ii) storage at -20ºC with 0.1% of EDTA and iii) pre-concentration in a solid phase extraction cartridge (SPE), which was afterwards stored at -20ºC. Concentrations of antibiotics in the samples preserved using the different protocols were monitored after 0, 1, 2 and 12 weeks. The results showed that, for the accurate determination of all compounds they should be analysed right after sampling. However, if this is not possible, most of the antibiotics can be analysed within the 1st week after sampling and preservation at -20ºC (with or without EDTA) or in a SPE cartridges at -20ºC. Nonetheless, some antibiotics found extensively in the environment, such as sulfamethoxazole, ciprofloxacin, ofloxacin, erythromycin, azithromycin and clarithromycin exhibited low stability after 1 week preservation and, therefore, they should be analysed within this time.

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Introduction

Antibiotics are a group of pharmaceuticals of current concern because of their high consumption and pseudo-persistence in the environment [1]. In addition, it is suspected that the chronic exposure to antibiotics could induce the development of antibiotic-resistant pathogens [2-5], which might be a case of alarm because of the subsequent impact in biota and human health. The presence of antibiotics in different environmental compartments including water, soil and biota, has been studied during the last years [6]. The availability of accurate and sensitive analytical methods to detect and quantify these compounds is crucial to address many of the environmental questions raised by their occurrence in the environment. Nowadays, liquid-chromatography methods coupled to mass spectrometry in tandem (LC-MS/MS) is the chosen technique for the analysis of antibiotics since it allows the detection of a wide number of compounds in just one run due to their high selectivity and sensitivity achieved using the selected reaction monitoring (SRM) acquisition mode [1, 6]. In this sense, some authors have developed during the last years fast multi-residue analytical methods for the analysis of a broad range of antibiotics in water [7, 8]. However, in most of the cases, the traceability of the analytes during sampling procedure, sample shipment and preservation are not studied in depth during method development. These aspects are very important since, in some occasions, immediate analysis of the samples is not possible and samples have to be kept for a while before they are analysed [9]. Some antibiotics, such as penicillins, cephalosporines and tetracyclines are high unstable and, therefore, stability of antibiotics during sample storage need to be assessed in order to ensure the veracity of the final analytical results [10-15]. For example, Gaugain *et al.* assessed the stability of some antibiotics in standard solutions, preserved at -18ºC in their optimum solvent, and in real matrix (cow milk and pork muscle tissue) at -18ºC and -70ºC [16]. The authors observed that stabilities in standard solution at -18ºC ranged from 1 to 6 months for lincosamides, cyclines, penicillins and cephalosporines and between 6 and 12 months for quinolones and sulfonamides [16]. In contrast, the stabilities were much higher in real matrix, close to 12 months for almost all tested antibiotics when samples were preserved at -70ºC [16]. Similar results were observed for (fluoro)quinolones in pig kidney samples where antibiotics were stable along 7 weeks when the samples were preserved at -20ºC [17]. However, there is a lack of information regarding the stability of antibiotics in water samples. In one of the few existing studies, the authors investigated the stability of sulfamethoxazole (among other chemical compounds) in spiked tap and river water samples [18]. The results showed that the response of this compound increases a little bit when the samples are preserved at 4ºC for 1 and 3 weeks after sampling [18].

The objective of this study was to evaluate different water sample preservation procedures prior to the analysis of 56 selected antibiotics and some of their metabolites. The wide set of antibiotics includes: macrolides (7), tetracyclines (4), fluoroquinolones (10), quinolones (4), penicillins (6), cephalosporines (6), lincosamides (2), sulfonamides (15) and nitroimidazole antibiotics (2).Three different preservation methodologies were proposed based on the most

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common ones reported in the literature [9]: i) to store samples at -20°C, ii) to store samples at -20ºC with the addition of 0.1% of EDTA and, finally, iii) to preserve samples by loading them into solid phase extraction (SPE) cartridges and stored at -20°C.

1. Materials and methods

2.1 Materials

Standards of target compounds (Table 1) were of high purity grade (>90%) and purchased from Sigma-Aldrich. All the solvents used were of high purity grade, supplied by Merck (Darmstadt, Germany), and ethylenediaminetetraacetic acid disodium salt (EDTA) and hydrochloric acid (HCl) concentrate were supplied by Panreac. Finally, solid phase extraction cartridges Oasis HLB (60 mg 3 ml) were purchased from Waters Corporation (Miltford, MA, U.S.A).

Each antibiotic was previously diluted in methanol, with a final concentration of 1000 µg/ml and kept at -20ºC. Then, the mix solution was prepared at 1 µg/ml in HPLC grade water for spiking purposes.

2.2 Methodology

The experiments were carried out by fortifying deionised water with a mixture of the 56 antibiotics at a final concentration of 10 ng/ml, covering the highest concentrations detected in a previous work [7].The use of fortified materials is accepted when there are no incurred materials available according to 2002/657/EC [19]. In parallel, blank samples were prepared with non-spiked deionised water in order to rule out any possible cross contamination during the process. The experiments were performed in triplicate for each preservation mode (-20ºC, -20ºC with 0.1% of EDTA and into a SPE cartridge kept at -20ºC) and thus three samples were taken at 5 different sampling times (after 0, 1, 2, 12 and 24 weeks) and analysed the same day by LC-MS/MS according to Gros *et al.* [7]. In total, 45 spiked samples and 45 blanks were collected in amber polypropylene bottles (20 ml water in each bottle).

A first set of 30 samples (15 spiked and 15 blanks) were prepared for their preservation at -20ºC: 24 of these samples (12 spiked and 12 blanks) were kept at -20ºC until analysis whereas the other 6 (3 spiked and 3 blanks) were analysed within the same day by LC-MS/MS [7] . For the direct analysis, 0.5 ml of the sample was introduced into LC-vial with 0.5 ml of methanol and then spiked with 10 µl of labelled antibiotics (internal standards) in methanol for a final concentration of 10 ng/ml [7]. The same procedure was followed for the rest of the samples at each sampling time. Another set of 30 water samples (15 spiked and 15 blanks) were preserved with EDTA (final concentration of 0.1%) before storage at -20ºC. 3 blanks and 3 spiked samples corresponding to time 0 were directly analysed as described for the samples preserved at -20ºC. Finally, the last set of 30 samples was extracted by SPE according to Gros *et al.* [7]. Briefly, 0.4 ml of EDTA at 5% was added to 20 ml of sample (final concentration of 0.1%) and pH adjusted to 2.5 with HCl. Then, the samples were homogenized for 30 min in an orbital

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106 digester, followed by SPE extraction [7] and finally preserved at -20 ºC. At the corresponding
107 sampling time, cartridges were thawed and eluted, reduced to dryness under N_2 stream and,
108 finally, reconstituted in a LC-vial with 0.5 ml of water and 0.5 ml of methanol [7]. 10 µl of labelled
109 antibiotics in methanol (100 ng/ml) were added as internal standards and finally analysed by
110 LC-MS/MS [7].

111 Percentage of remaining antibiotic concentration in water samples was calculated as follows:

112 $$\%\ Remaining\ Antibiotic = \frac{(Peak\ Area)_i\ at\ X\ weeks\ /\ Peak\ Area\ of\ IS\ in\ sample}{(Peak\ Area)_i\ at\ 0h\ /\ Peak\ Area\ of\ IS\ in\ sample\ at\ 0h} \times 100,$$

113 where “Peak Area” corresponds to the chromatographic area after LC-MS/MS analysis of
114 antibiotic “i” and the corresponding internal standard (IS) in this sample. In addition, the
115 concentration of the compounds was calculated by external calibration curve at each sampling
116 point in order to monitor the response of the instrument along the time. The standard calibration
117 curves of antibiotics were prepared the same day of the analysis in order to avoid any
118 degradation of the stock solutions, which are stable for the selected antibiotics for more than 6
119 months in methanol [16].

120 In addition, the pH of the samples tested at the beginning of the experiment as well as at each
121 sampling time. This was maintained around 8 without drastic changes between blanks and
122 spiked experiments along sampling times.

123

124 ## 2. Results and discussion

125 An example of chromatograms is presented in Figures 1 and 2 and all the results about the
126 remaining antibiotics at each sampling time and for each type of preservation method are
127 summarized in Figures 3 to 5. Antibiotics were considered unstable when the remaining
128 antibiotic percentage was below 80% according to Hillebrand *et al.* [11]. The report of stable
129 compounds is presented in Table 2.

130 The results indicated, as expected, that antibiotics exhibit different stability depending on their
131 chemical group. In this sense, pencicillins, cephalosporines, sulfonamides, nitroimidazoles and
132 lincosamides can be considered as relatively stable (loss of compounds were not higher than
133 20% after three months preservation) whereas fluoroquinolones, quinolones, tetracyclines and
134 macrolides are the most unstable groups of the studied antibiotics (Table 2).

135 The nitroimidazole compounds investigated showed the most stable profile with more than 80%
136 of the initial compound after 12 weeks of preservation, independent of the preservation
137 conditions (Figure 3 and Table 2). In the case of penicillins, these compounds remained stable
138 along the whole experiment with the exception of Ampicillin (with a decrease higher than 20% in
139 all preservation modes (Figure 3)) and are better preserved at -20ºC with and without addition of

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EDTA. However, the results observed by Gaugain *et al.* [16] during their investigation about the stability of the standards preserved in water:methanol (1:1) at -18ºC showed that this compound was stable in the solvent for 31 days. Nonetheless, we consider that this compound must be analysed within the first 2 weeks after sampling, in all the preservation modes tested (Table 2).

Regarding the 15 sulfonamides included in this study, neither the preservation at -20ºC nor the extraction into SPE cartridges improved the stability of these compounds along time compared to the storage of water samples at -20ºC with EDTA agent. Therefore, the latest was the most stable preservation mode for sulfonamides, whose loss were lower than 20% up to 12 weeks with the exception of sulfamethoxazole, sulfathiazole, sulfaperoxypiridazine and sulfisoxazole (Figure 4 and Table 2). In contrast, Gawlik *et al.* [18] observed a slight increase in sulfamethoxazole concentration in spiked river waters after 3 weeks.

Although lincosamides and cephalosporines are also quite stable compounds, a loss higher than 20% was observed for some of them between 0-12 weeks (Figures 2 and 3, respectively, and Table 2). For example, lincomycin exhibited a decrease near to 40% when the preservation was into SPE cartridge while this decrease reached the 30% when the samples were just kept at -20ºC with or without EDTA at -20ºC for 12 weeks. Nonetheless, the best preservation procedure for these two groups was the addition of EDTA agent followed by sample storage at -20ºC. Nevertheless, analysis of the samples is recommended to be performed within the first 7 days after sampling.

Finally, quinolones, fluoroquinolones, tetracyclines and macrolides were the most unstable groups (Figures 3 and 5 and Table 2). In the case of quinolones, the preservation into SPE cartridge at -20ºC increased the stability of the compounds although a loss higher than 20% was observed after the 1st week. However, the conservation of the samples at -20ºC without any additive could be recommended if the analysis is performed within the first 7 days after sampling (Figure 3). A similar pattern was observed for fluoroquinolones. The addition of EDTA implies a drastic decrease of the stability while the preservation at -20ºC, or into SPE cartridge were the most stable choices. Nevertheless, a loss higher than 40% was observed in both cases after 2 weeks (Figure 5 and Table 2). For macrolides, a slightly higher stability was observed when the samples were preserved into SPE cartridge at -20ºC, at least for erythromycin, azithromycin, tylosin, clarithromycin and roxithromycin, although losses were still *c.a.* 25% after one week storage (Figure 5 and Table 2). Finally, no differences were found for tetracyclines in the different preservation modes and the analysis is recommended to be carried out within the first 7 days after sampling (with samples preserved at -20ºC before analysis) (Figure 5).

At this point, it is important to notice that, although the experiments were prepared to test the stability for 24 weeks, the stability experiments were stopped after 12 weeks since the percentage of remaining antibiotic was very low for macrolides, tetracyclines and fluoroquinolones.

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177 37 compounds out of the 56 target antibiotics were stable after one week storage under, at
178 least, one of the preservation strategies tested (see Table 2). The number of stable compounds
179 decreased up to 32 compounds after 2 weeks whereas only 22 compounds were still stable
180 after the longest period of time tested (12 weeks).

181 Among the whole set of antibiotics assessed in the stability study, 12 compounds –
182 metronidazole, metronidazole-OH, cefazolin, cefalexin, cefotaxime, cetiofur, sulfamethoxazole,
183 ciprofloxacine, ofloxacine, erythromycin, azithromycin and clarithromycin- are found with the
184 highest frequency and at the highest levels in environmental waters as reported in the literature
185 [7, 20-22]. Within them, the 2 nitroimidazole and 4 cephalosporines exhibited sufficient stability
186 with at least one of the preservation modes tested along all preservation times, whereas the
187 stability of the other 6 relevant antibiotics can only be assured during the first week of storage,
188 with the exception of erythromycin whose concentration decreased lower than 80% in the first
189 week with all the preservation protocols. Erythromycin is, in fact, included in the Contaminant
190 Candidate List 3 for drinking water monitoring by the American Environmental Protection
191 Agency [23]. Despite of their poor stability, these 6 compounds (sulfamethozazole,
192 ciprofloxacine, ofloxacine, erythromycin, azithromycin and clarithromycin) are constantly
193 detected in environmental waters and at high concentrations. Nevertheless, particular care has
194 to be taken for the best preservation of the samples to assure accurate analysis and to avoid
195 underestimation of their presence and impact in the environment.

196 According to the results here discussed, selection of the best sample preservation will depend
197 on the group of antibiotics to be studied or analysed in each particular case. Even though the
198 analysis right after sample collection is the best option, most of the antibiotics can be analysed
199 within the 1st week after sampling and preservation of the samples at -20ºC (with or without
200 EDTA) or in a SPE cartridges at -20ºC. Another alternative could be to add the isotopically
201 labelled compound for each analyte (the so-called surrogate internal standards) just after
202 sampling and before analysis, in order to monitor any possible loss during storage and
203 normalize the final results. This is the approach proposed by Carlson *et al.* [15] for example,
204 who applied it for the analysis of a bunch of polar pollutants. This strategy is limited by the
205 availability of commercial isotopically labelled compounds for each of the analytes tested, as it
206 was the case in our study. Other techniques like direct injection have been proved to be useful
207 allowing shorter storage times and reducing costs [24]. Nonetheless, this last one is limited due
208 to the high sensitivity of the instruments required.

209 ## 3. Conclusions

210 The stability test here presented showed that, for this three months study in deionised water,
211 pencicillins, cephalosporines, sulfonamides, nitroimidazoles and lincosamides can be
212 considered as relatively stable while fluoroquinolones, quinolones, tetracyclines and macrolides
213 are the most unstable groups of the studied antibiotics. The latest include ofloxacin,

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ciprofloxacin, azithromycin, clarithromycin and erythromycin which, despite their low stability during storage, are still some of the most detected compounds in environmental samples.

Because of the different stability patterns observed depending on the group of antibiotics, the best option in multi-residue analytical methods is the analysis of the samples immediately after sampling. The analysis can be done within the1st week for the majority of the antibiotics if the samples are preserved with any of the preservation methods tested.

The preservation of antibiotics in water can be very problematic and should be considered carefully before sampling and analysis. However, a more extensive study considering environmental water should be done in order to assess the stability of selected analytes in more complex real matrices.

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Figure capitations:

Figure 1: Example of extracted ion chromatograms of target antibiotics in the samples preserved at -20ºC, with EDTA at -20ºC and in a SPE cartridge at -20ºC, at 0 and 1 weeks sampling.

Figure 2: Example of extracted ion chromatograms of target antibiotics in the samples preserved at -20ºC, with EDTA at -20ºC and in a SPE cartridge at -20ºC, at 2 and 12 weeks sampling.

Figure 3: Relative recovery percentage of antibiotics preserved at -20°C (n=3), with EDTA at -20ºC (n=3) and in a SPE cartridge at -20ºC for **A)** Penicillins, **B)** Nitroimidazole antibiotics, **C)** Lincosamides and **D)** Quinolones. 0 h (t0),1 week (t1), 2 weeks (t2), 12 weeks (t3).

Figure 4: Relative recovery percentage of antibiotics preserved at -20°C (n=3), with EDTA at -20ºC (n=3) and in a SPE cartridge at -20ºC for **A)** Sulfonamides and **B)** Cephalosporines. 0 h (t0), 1 week (t1), 2 weeks (t2), 12 weeks (t3).

Figure 5: Relative recovery percentage of antibiotics preserved at -20°C (n=3), with EDTA at -20ºC (n=3) and in a SPE cartridge at -20ºC for **A)** Fluoroquinolones, **B)** Tetracyclines and **C)** Macrolides. 0 h (t0), 1 week (t1), 2 weeks (t2), 12 weeks (t3).

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319 Table 1: list of antibiotics studied during the stability tests.

Chemical group	Compound	Molecular formula	Chemical group	Compound	Molecular formula
Macrolides	Erythromycin	$C_{37}H_{67}NO_{13}$	Cephalosporines	Cefazolin	$C_{14}H_{13}N_8O_4S_3$
	Azithromycin	$C_{38}H_{72}N_2O_{12}$		Cefuroxime	$C_{16}H_{16}N_4O_8S$
	Tilmicosin	$C_{46}H_{80}N_2O_{13}$		Cefapirin	$C_{17}H_{16}N_3O_6S_2$
	Tylosin	$C_{46}H_{77}NO_{17}$		Cefalexin	$C_{16}H_{17}N_3O_4S$
	Clarithromycin	$C_{38}H_{69}NO_{13}$		Cefotaxime	$C_{16}H_{16}N_5O_7S_2$
	Roxithromycin	$C_{41}H_{76}N_2O_{15}$		Cetifour	$C_{19}H_{17}N_5O_7S_3$
	Spiramycin	$C_{43}H_{74}N_2O_{14}$			
			Lincosamides	Clindamycin	$C_{18}H_{33}ClN_2O_5S$
Tretracyclines	Tcetracycline	$C_{22}H_{24}N_2O_8$		Lincomycin	$C_{18}H_{34}N_2O_6S$
	Doxycycline	$C_{22}H_{24}N_2O_8$			
	Chlorotetracycline	$C_{22}H_{23}ClN_2O_8$	Sulfonamides	Sulfamethoxazole	$C_{10}H_{11}N_3O_3S$
	Oxytetracycline	$C_{22}H_{24}N_2O_9$		Sulfisomidin	$C_{12}H_{14}N_4O_2S$
				Sulfadiazine	$C_{10}H_{10}N_4O_2S$
Fluoroquinolones	Ofloxacin	$C_{18}H_{20}FN_3O_4$		Sulfamerazine	$C_{11}H_{12}N_4O_2S$
	Ciprofloxacin	$C_{17}H_{18}N_3FO_3$		Sulfathiazole	$C_9H_9N_3O_2S_2$
	Enrofloxacin	$C_{19}H_{22}FN_3O_3$		Sulfapyridine	$C_{11}H_{11}N_3O_2S$
	Danofloxacin	$C_{19}H_{20}FN_3O_3$		Sulfabenzamide	$C_{13}H_{12}N_2O_3S$
	Orbifloxacin	$C_{19}H_{20}F_3N_3O_3$		Sulfadimethoxine	$C_{12}H_{14}N_4O_4S$
	Marbofloxacin	$C_{17}H_{19}FN_4O_4$		Sulfamethizole	$C_9H_{10}N_4O_2S_2$
	Cinoxacin	$C_{12}H_{10}N_2O_5$		Sulamethoxypiridazine	$C_{11}H_{12}N_4O_3S$
	Norfloxacin	$C_{16}H_{18}FN_3O_3$		Sulfisoxazole	$C_{11}H_{13}N_3O_3S$
	Difloxacin	$C_{21}H_{19}F_2N_3O_3$		Sulfanitran	$C_{14}H_{13}N_3O_5S$
	Enoxacin	$C_{15}H_{17}FN_4O_3$		N-acetylsulfadiazine*	$C_{12}H_{12}N_4O_3S$
				N-acetylsulfamethazine*	$C_{14}H_{16}N_4O_3S$
Quinolones	Flumequine	$C_{14}H_{12}FNO_3$		N-acetylsulfamerazine*	$C_{13}H_{14}N_4O_3S$
	Nalidixic acid	$C_{12}H_{12}N_2O_3$			
	Pipemidic acid	$C_{14}H_{17}N_5O_3$	Nitroimidazole antibiotics	Metronidazole-OH*	$C_6H_9N_3O_4$
	Oxolinic acid	$C_{13}H_{11}NO_5$		Metronidazole	$C_6H_9N_3O_3$
Penicillins	Amoxicillin	$C_{16}H_{19}N_3O_5S$			
	Ampicillin	$C_{16}H_{19}N_3O_4S$			
	Penicillin G	$C_{16}H_{17}N_2O_4S$			
	Penicillin V	$C_{16}H_{17}N_2O_5S$			
	Cloxacillin	$C_{19}H_{18}ClN_3O_5S$			
	Oxacillin	$C_{19}H_{18}N_3O_5S$			

320 *Metabolites

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322 Table 2: Percentage of remaining antibiotics after corresponding sample preservation. The 12
323 most frequently detected antibiotics in different water samples are highlighted in grey [7, 20-22].

	-20°C			0.1%EDTA			SPE		
	1 week	2 weeks	12 weeks	1 week	2 weeks	12 weeks	1 week	2 weeks	12 weeks
Macrolides									
Erythromycin	< 80%	< 80%	< 80%	< 80%	< 80%	< 80%	< 80%	< 80%	< 80%
Azithromycin	< 80%	< 80%	< 80%	< 80%	< 80%	< 80%	**Stable**	< 80%	< 80%
Tilmicosin	< 80%	< 80%	< 80%	< 80%	< 80%	< 80%	< 80%	< 80%	< 80%
Tylosin	< 80%	< 80%	< 80%	< 80%	< 80%	< 80%	**Stable**	< 80%	< 80%
Clarithromycin	< 80%	< 80%	< 80%	< 80%	< 80%	< 80%	**Stable**	< 80%	< 80%
Roxithromycin	< 80%	< 80%	< 80%	< 80%	< 80%	< 80%	**Stable**	< 80%	< 80%
Spiramycin	< 80%	< 80%	< 80%	< 80%	< 80%	< 80%	< 80%	< 80%	< 80%
Tretracyclines									
Tcetracycline	< 80%	< 80%	< 80%	< 80%	< 80%	< 80%	< 80%	< 80%	< 80%
Doxycycline	< 80%	< 80%	< 80%	< 80%	< 80%	< 80%	< 80%	< 80%	< 80%
Chlorotetracycline	< 80%	< 80%	< 80%	< 80%	< 80%	< 80%	< 80%	< 80%	< 80%
Oxytetracycline	< 80%	< 80%	< 80%	< 80%	< 80%	< 80%	< 80%	< 80%	< 80%
Fluoroquinolones									
Ofloxacin	< 80%	< 80%	< 80%	**Stable**	< 80%	< 80%	< 80%	< 80%	< 80%
Ciprofloxacin	< 80%	< 80%	< 80%	**Stable**	< 80%	< 80%	< 80%	< 80%	< 80%
Enrofloxacin	**Stable**	< 80%	< 80%	< 80%	< 80%	< 80%	**Stable**	< 80%	< 80%
Danofloxacin	< 80%	< 80%	< 80%	< 80%	< 80%	< 80%	**Stable**	< 80%	< 80%
Orbifloxacin	**Stable**	< 80%	< 80%	< 80%	< 80%	< 80%	**Stable**	< 80%	< 80%
Marbofloxacin	**Stable**	< 80%	< 80%	< 80%	< 80%	< 80%	< 80%	< 80%	< 80%
Cinoxacin	< 80%	< 80%	< 80%	**Stable**	**Stable**	< 80%	**Stable**	< 80%	< 80%
Norfloxacine	**Stable**	< 80%	< 80%	< 80%	< 80%	< 80%	< 80%	< 80%	< 80%
Difloxacin	**Stable**	**Stable**	< 80%	< 80%	< 80%	< 80%	< 80%	< 80%	< 80%
Enoxacin	< 80%	< 80%	< 80%	< 80%	< 80%	< 80%	< 80%	< 80%	< 80%
Quinolones									
Flumequine	< 80%	< 80%	< 80%	< 80%	< 80%	< 80%	< 80%	< 80%	< 80%
Nalidixic acid	**Stable**	**Stable**	< 80%	**Stable**	**Stable**	**Stable**	**Stable**	< 80%	< 80%
Pipemidic acid	**Stable**	**Stable**	< 80%	< 80%	< 80%	< 80%	< 80%	< 80%	< 80%
Oxolinic acid	**Stable**	< 80%	< 80%	< 80%	< 80%	< 80%	**Stable**	< 80%	< 80%
Penicillins									
Amoxicillin	**Stable**	**Stable**	**Stable**	**Stable**	**Stable**	**Stable**	**Stable**	**Stable**	**Stable**
Ampicillin	< 80%	< 80%	< 80%	**Stable**	< 80%	**Stable**	**Stable**	< 80%	< 80%
Penicillin G	**Stable**	**Stable**	**Stable**	**Stable**	**Stable**	**Stable**	**Stable**	< 80%	**Stable**
Penicillin V	**Stable**	**Stable**	**Stable**	**Stable**	**Stable**	**Stable**	**Stable**	< 80%	**Stable**
Cloxacillin	**Stable**	**Stable**	**Stable**	**Stable**	**Stable**	**Stable**	**Stable**	< 80%	**Stable**
Oxacillin	**Stable**	**Stable**	**Stable**	< 80%	< 80%	**Stable**	**Stable**	< 80%	**Stable**
Cephalosporines									
Cefazolin	**Stable**	**Stable**	**Stable**	**Stable**	**Stable**	**Stable**	**Stable**	< 80%	**Stable**
Cefuroxime	< 80%	< 80%	< 80%	**Stable**	**Stable**	**Stable**	**Stable**	< 80%	< 80%
Cefapirin	< 80%	**Stable**	**Stable**	**Stable**	**Stable**	**Stable**	**Stable**	< 80%	< 80%
Cefalexin	**Stable**	**Stable**	**Stable**	**Stable**	**Stable**	**Stable**	**Stable**	< 80%	**Stable**
Cefotaxime	< 80%	**Stable**	< 80%	< 80%	**Stable**	< 80%	< 80%	< 80%	< 80%
Cetifour	**Stable**	**Stable**	**Stable**	**Stable**	**Stable**	**Stable**	**Stable**	< 80%	**Stable**
Lincosamides									
Clindamycin	**Stable**	**Stable**	< 80%	**Stable**	**Stable**	< 80%	< 80%	< 80%	< 80%
Lincomycin	< 80%	< 80%	< 80%	**Stable**	**Stable**	< 80%	< 80%	< 80%	< 80%
Sulfonamides									
Sulfamethoxazole	< 80%	< 80%	< 80%	**Stable**	< 80%	< 80%	< 80%	< 80%	< 80%
Sulfisomidin	**Stable**	**Stable**	< 80%	**Stable**	**Stable**	**Stable**	**Stable**	< 80%	< 80%
Sulfadiazine	< 80%	< 80%	< 80%	**Stable**	**Stable**	**Stable**	**Stable**	< 80%	< 80%
Sulfamerazine	**Stable**	**Stable**	< 80%	**Stable**	**Stable**	**Stable**	**Stable**	**Stable**	**Stable**
Sulfathiazole	**Stable**	**Stable**	< 80%	**Stable**	**Stable**	< 80%	**Stable**	**Stable**	**Stable**
Sulfapyridine	**Stable**	**Stable**	**Stable**	**Stable**	**Stable**	**Stable**	**Stable**	**Stable**	**Stable**
Sulfabenzamide	**Stable**	**Stable**	< 80%	**Stable**	**Stable**	< 80%	**Stable**	< 80%	< 80%
Sulfadimethoxine	**Stable**	**Stable**	< 80%	**Stable**	**Stable**	< 80%	**Stable**	< 80%	< 80%
Sulfamethizole	**Stable**	**Stable**	**Stable**	**Stable**	**Stable**	**Stable**	**Stable**	< 80%	< 80%
Sulamethoxypiridazine	**Stable**	**Stable**	< 80%	**Stable**	**Stable**	< 80%	**Stable**	< 80%	< 80%
Sulfisoxazole	< 80%	< 80%	< 80%	< 80%	< 80%	< 80%	< 80%	< 80%	< 80%
Sulfanitran	**Stable**	**Stable**	**Stable**	**Stable**	**Stable**	**Stable**	**Stable**	< 80%	< 80%
N-acetylsulfadiazine	**Stable**	**Stable**	< 80%	**Stable**	**Stable**	< 80%	**Stable**	< 80%	< 80%
N-acetylsulfamethazine	**Stable**	**Stable**	**Stable**	**Stable**	**Stable**	**Stable**	**Stable**	**Stable**	**Stable**
N-acetylsulfamerazine	**Stable**	**Stable**	< 80%	**Stable**	**Stable**	**Stable**	**Stable**	< 80%	< 80%
Nitroimidazole antibiotics									
metronidazole-OH	**Stable**	**Stable**	**Stable**	**Stable**	**Stable**	**Stable**	**Stable**	**Stable**	**Stable**
metronidazole	**Stable**	**Stable**	**Stable**	**Stable**	**Stable**	**Stable**	**Stable**	**Stable**	**Stable**

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Frozen EDTA SPE

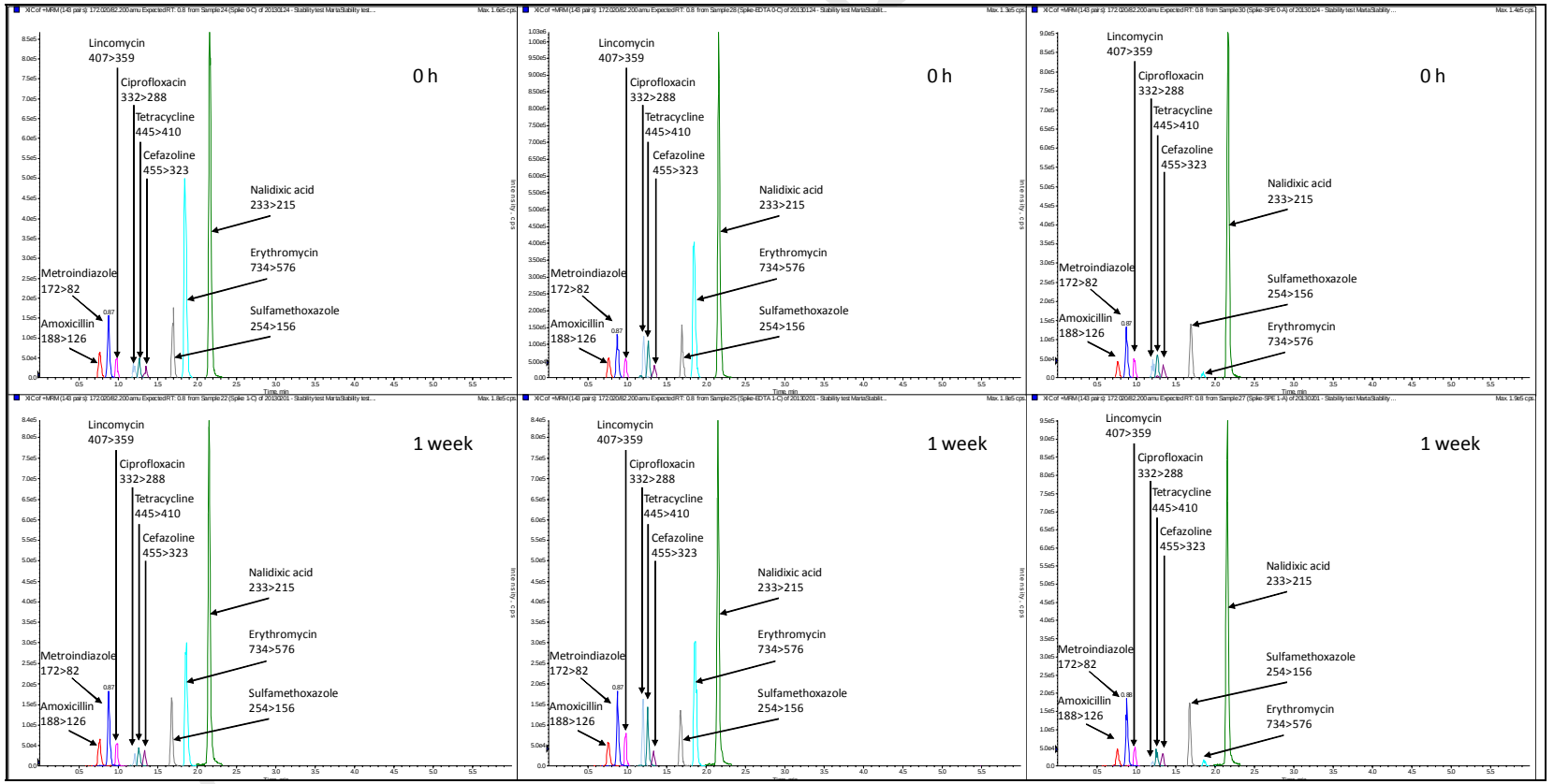

Figure 1

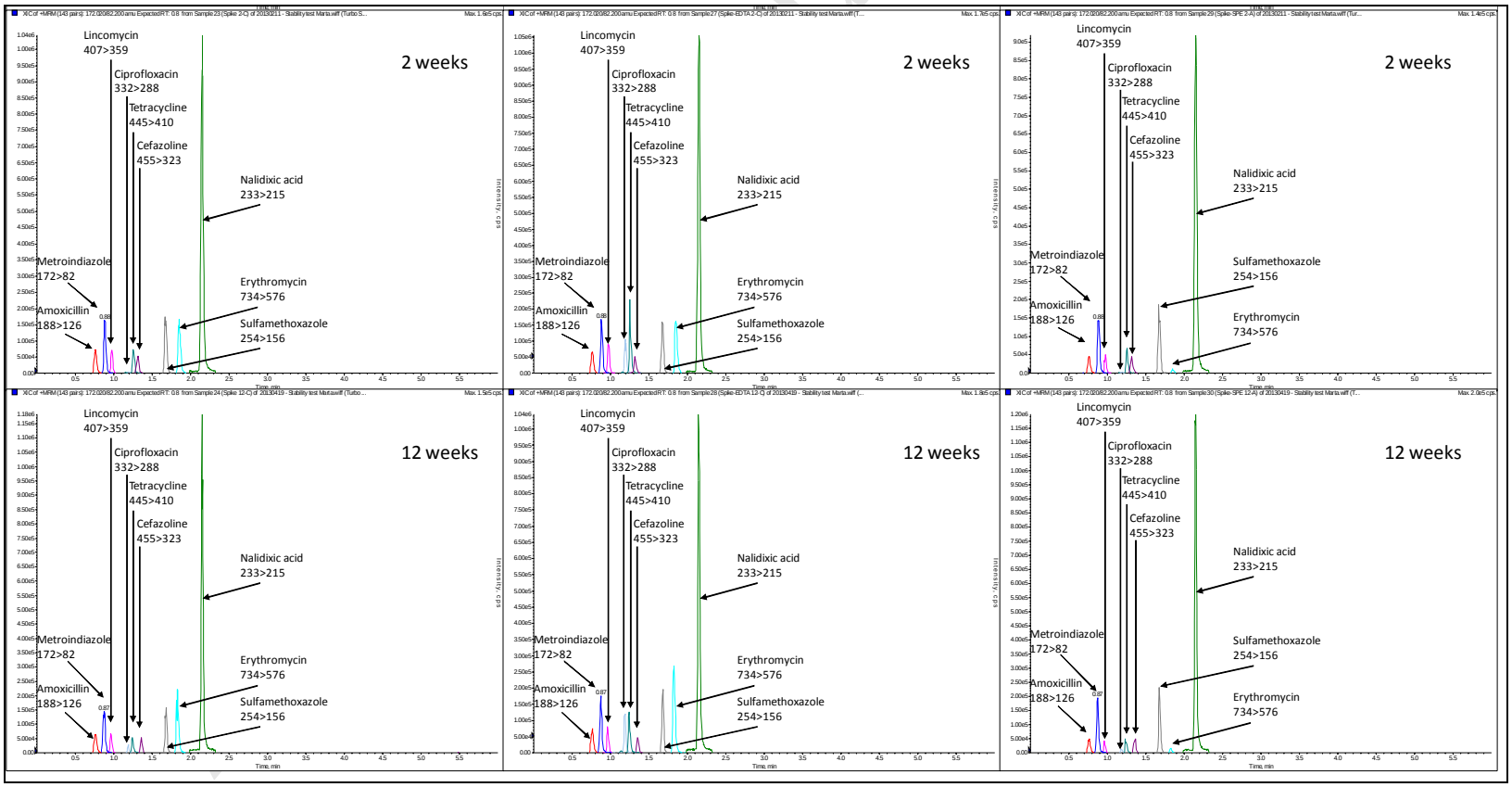
Frozen
EDTA
SPE
2 weeks
12 weeks
Lincomycin
407>359
Ciprofloxacin
332>288
Tetracycline
445>410
Cefazoline
455>323
Nalidixic acid
233>215
Metroindiazole
172>82
Amoxicillin
188>126
Erythromycin
734>576
Sulfamethoxazole
254>156
Intensity, cps
Time, min

Figure 2

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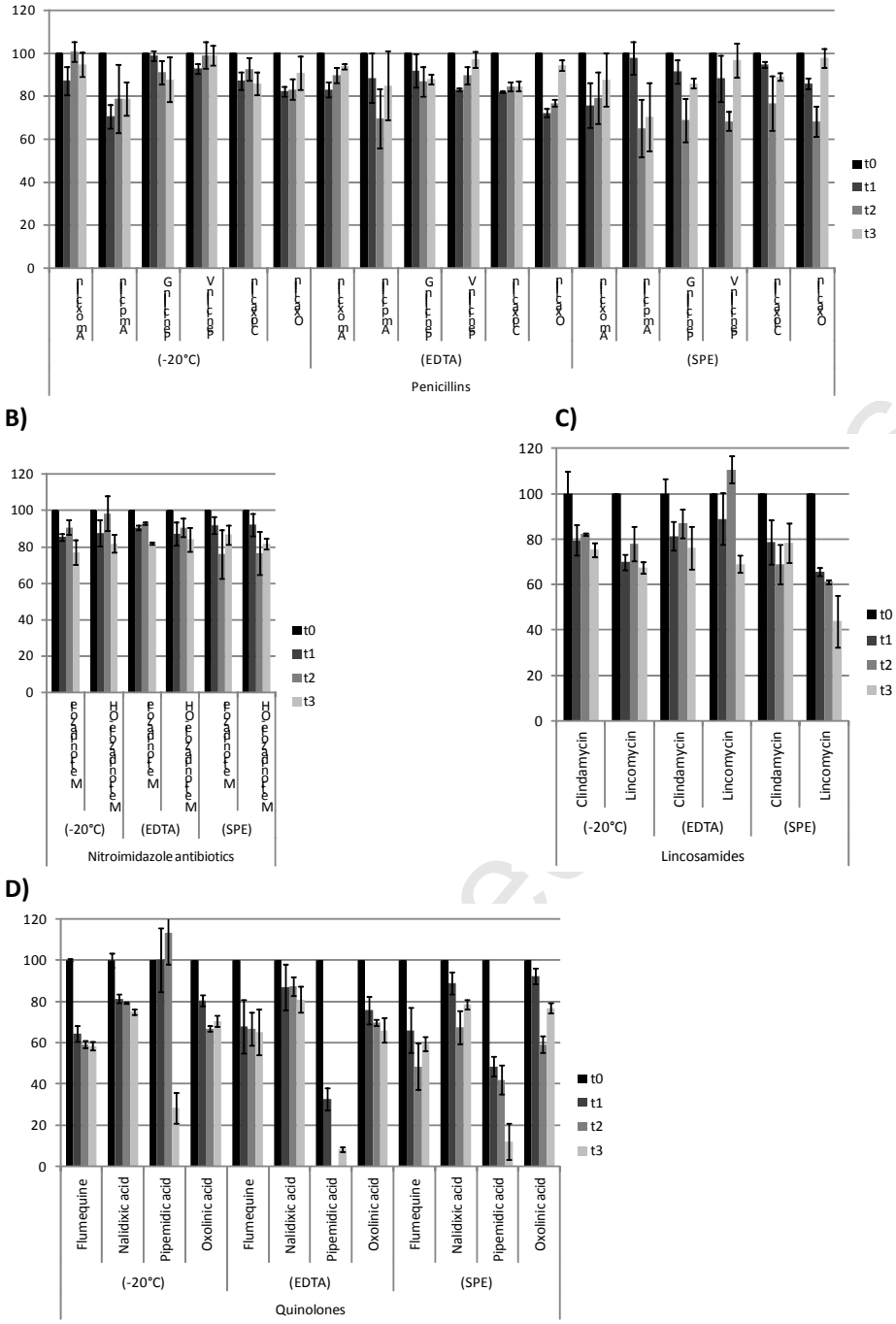
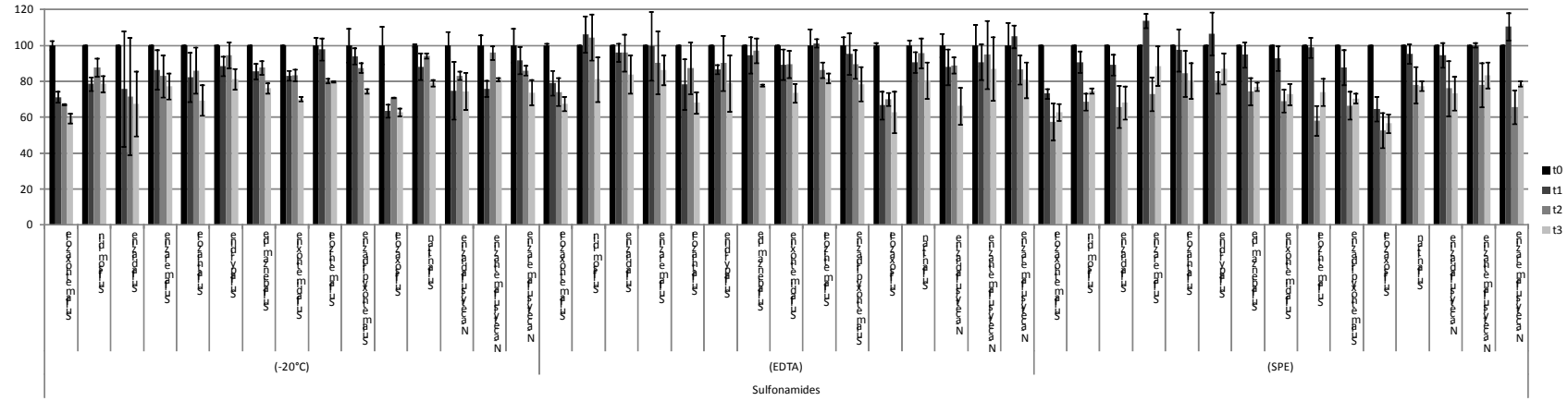


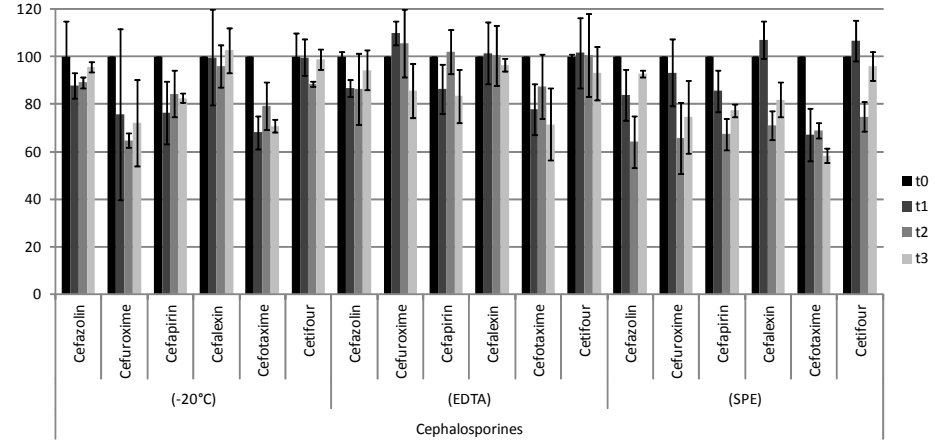
Figure 3

340 A)



341

342 B)



343

344 Figure 4

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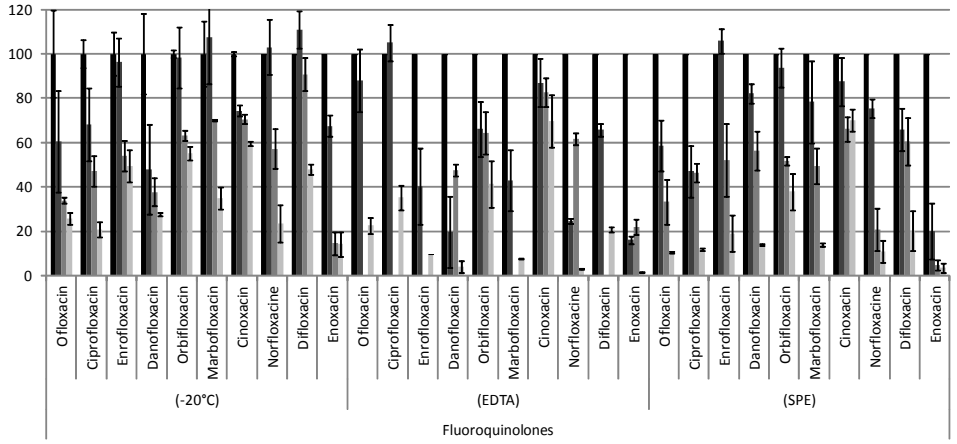
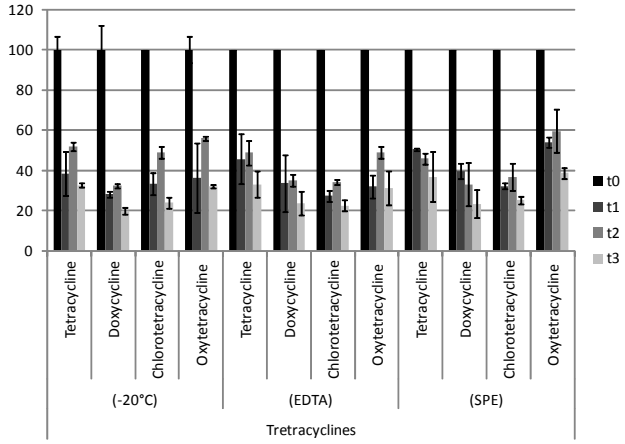
A)



B)



C)

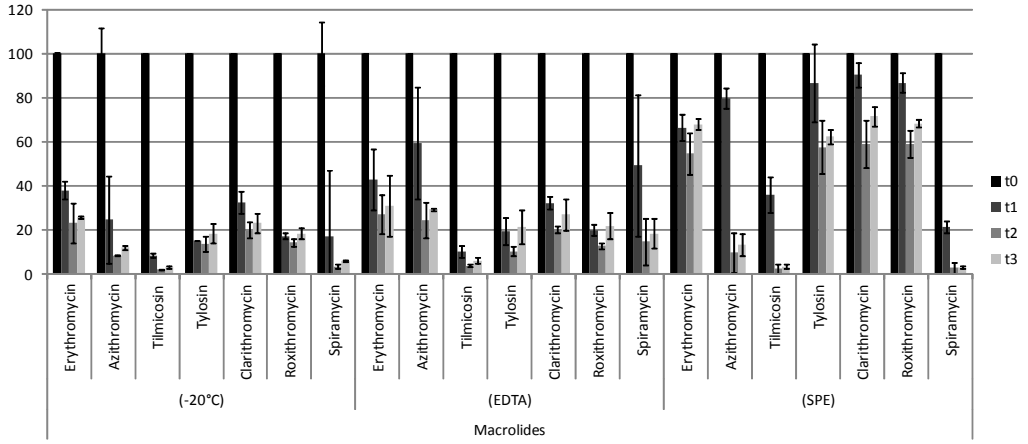


Figure 5

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Highlights:

- Antibiotics stability in water
- Preservation modes
- Liquid chromatography coupled to mass spectrometry in tandem

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