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Introduction

In 2015, an estimated 11,134.97 tonnes of antibiotics were consumed by humans in 59 countries. This figure is projected to increase to 128 billion doses by 2030. Antibiotics that are not fully metabolised by the organism end up in wastewater treatment plants. Conventional wastewater treatment plants cannot completely remove antibiotics, which remain in anaerobic treated effluents (ATEs). ATEs themselves lead to the occurrence of antibiotic resistance genes due to their interaction with microbes in treatment facilities. This establishes a clear link between antibiotic use and antibiotic resistance, which causes over 700,000 deaths per year. Thus, it is necessary to accurately measure antibiotic levels in the environment, but the precision of existing methods is limited by the occurrence of matrix effects. Matrix effects are an increase or decrease in the response of the target analyte, an effect which can dramatically alter the performance of an analytical method. At present, there is no method developed for the detection of antibiotics that is not subjected to inaccuracies due to matrix effects.

Sample Preparation Method

Liquid samples were vacuum filtered using a 0.45 µm membrane before separation. Solid samples were prepared by double extraction via centrifugation in 50 mL methanol. Both sample types were extracted and concentrated via Solid Phase Extraction (SPE), using Oasis HLB 6cc 500 µg SPE cartridges. Analytes were eluted using acetonitrile, and reconstituted in a mobile phase composed of 10:10:80 methanol:acetonitrile:water. SPE has been found to contribute to the presence of matrix effects¹. The possibility of removing the SPE step from the sample preparation and using an alternative reconcentration method was explored, but the application depends on the analyte concentrations observed in the samples.

LC-QQQ-MS Method

LC-MS/MS analysis was completed using an Agilent HPLC instrument with a 1290 Infinity II LC multi-sampler. The columns used were a Zorbax Eclipse Plus C18 2.1 x 50 mm 1.8 µm LC column, and a Zorbax Eclipse Plus C18 2.1 x 5 mm, 1.8 µm UHPLC guard column at 30°C. A 6470A triple-quadrupole mass spectrometer with electrospray ionization from Agilent was used for detection. He was used as a collision gas, and N₂ as a nebulising and desolvation gas.

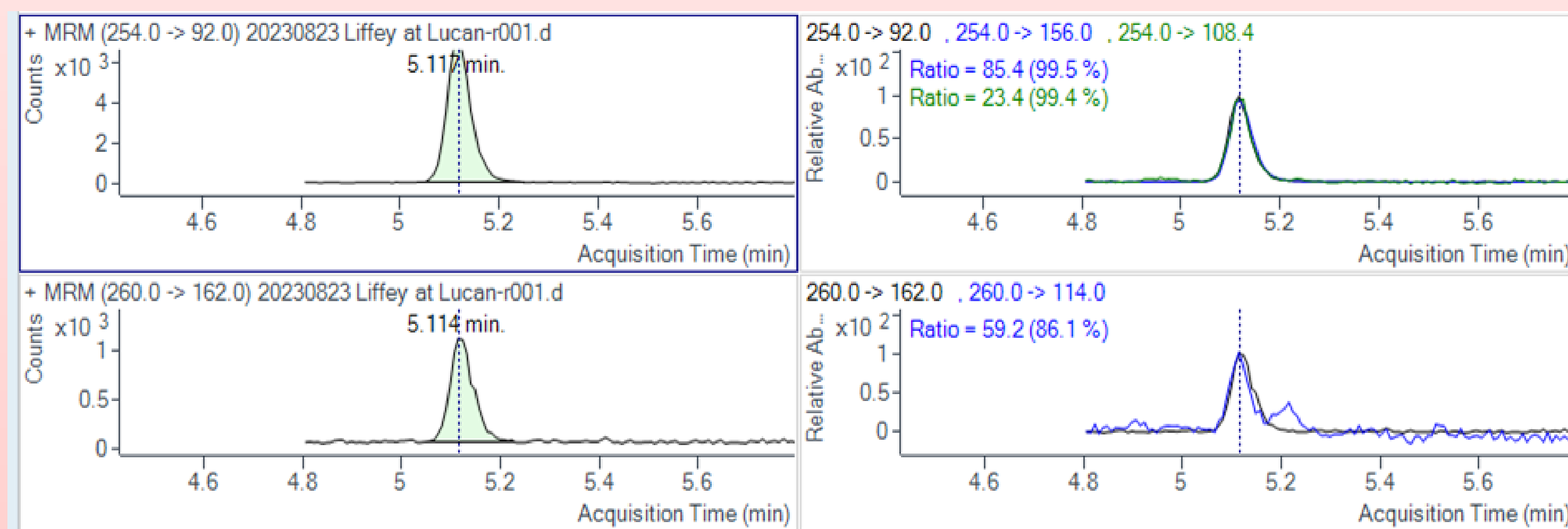


Figure 1 MRM scans of sulfamethoxazole obtained using the LC-QQQ-MS instrument. The analysed sample is an environmental water sample taken from the River Liffey, and analysed using the discussed SPE and LC-MS/MS methods.

Fragmentation & Degradation

Many antibiotic compounds degrade readily in the environment, be it due to temperature, pH or digestion by living organisms. While optimizing the LC-MS/MS method, the degradation pathways of the chosen antibiotics - including SMX, Figure 3 - in the environment were examined, and where possible, the degradation products were purchased as standards and included in the LC-MS method. This allows for the method to produce an accurate representation of antibiotic presence in the analysed samples.

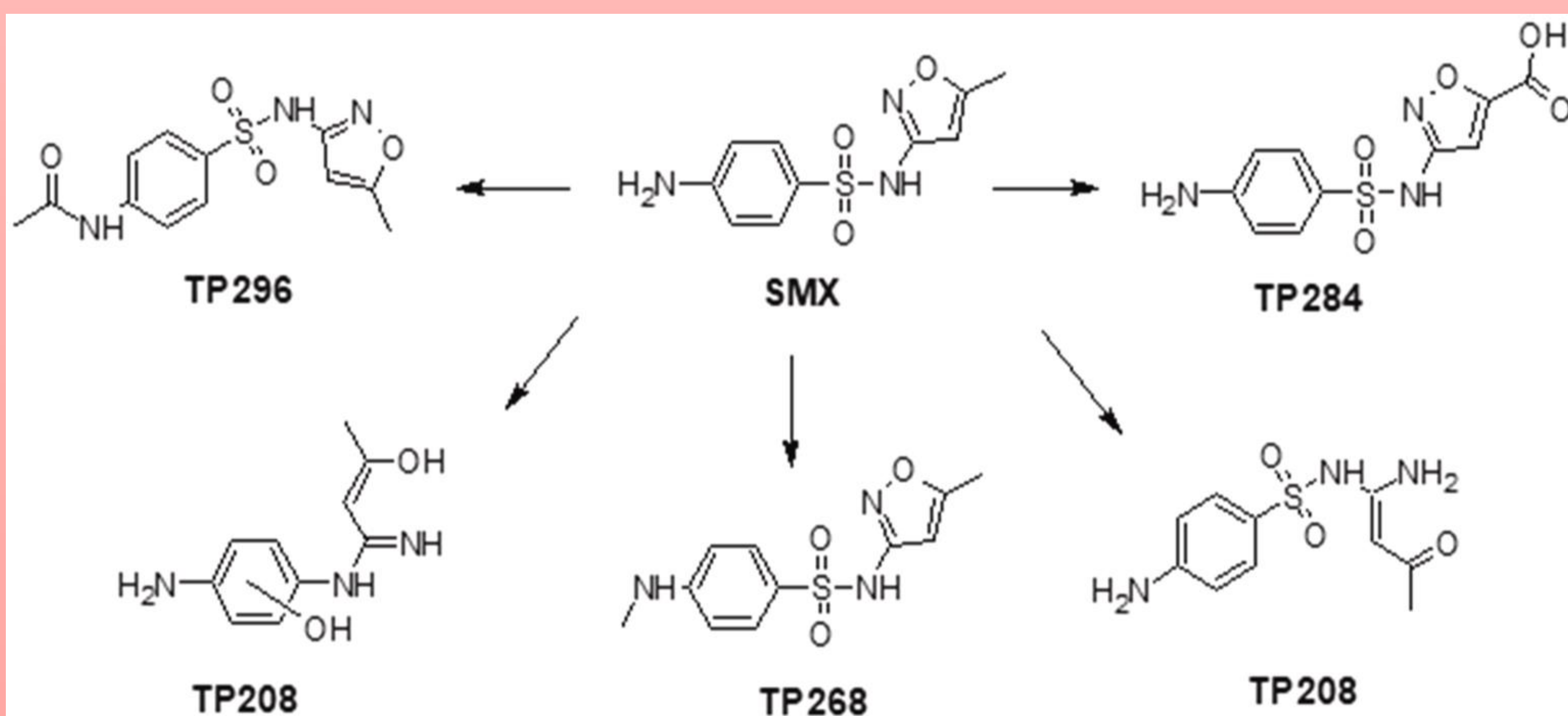


Figure 3 Degradation pathway of sulfamethoxazole (SMX) in the environment, into transformation products (TP). Where possible, these transformation products were purchased and included in the LC-MS/MS method.³

Acknowledgements and References

The authors would like to thank Science Foundation Ireland (SFI) and the Engineering and Physical Sciences Research Council (EPSRC) for funding this work. 1) Souverain S., Rudaz S., Veuthey J-L., Matrix effect in LC-ESI-MS and LC-APCI-MS with off-line and on-line extraction procedures (2004) J Chromatogr A, 1058(1-2):61-6. 2) Stockholm Convention on Persistent Organic Pollutants (POPs). Geneva: United Nations Environment Programme. 2018. pp. Annex D. 3) Kokoszka K., Wilk J., Felis E., Bajkacz S., Application of UHPLC-MS/MS method to study occurrence and fate of sulfonamide antibiotics and their transformation products in surface water in highly urbanized areas. (2021) Chemosphere 28

Antibiotics of Interest

Five antibiotics were chosen as compounds of interest, each representing a particular class of antibiotic. All five antibiotics are commonly used to treat humans and animals, and therefore readily present in environmental and waste waters. The selected antibiotics and their represented classes, as well as their CAS number and log K_{ow} can be found in Table 1. The log K_{ow} is used to assess the environmental fate of pollutants; compounds with a value greater than 5, for example, are more likely to accumulate in the fatty tissues of organisms (bioaccumulation)².

Selected Antibiotics			
Common Name	Antibiotic Class	CAS Number	Log K_{ow}
Ciprofloxacin (CPFX)	fluoroquinolones	85721-33-1	0.28
Amoxicillin (AMX)	aminopenicillins	26787-78-0	1.347
Tetracycline (TET)	macrolides	64-75-5	-1.37
Erythromycin (ERY)	sulfonamides	114-07-8	2.54
Sulfamethoxazole (SMX)	tetracyclines	723-46-6	0.89

Table 1 Antibiotics identified as compounds of interest, the class of antibiotics they represent, their CAS numbers and their log K_{ow} (octanol/water partition coefficient).

Sulfamethoxazole (SMX) Detection

One of the five chosen antibiotics, sulfamethoxazole (SMX), was found in 4 of the 5 investigated surface water bodies in Ireland (Figure 2) using existing sample preparation and LC-MS/MS methods. The lowest detected concentration was 0.65 ng/L in samples taken from the River Suir. This indicates the high sensitivity of this method and its suitability for use. While recovery values were satisfactory (87.83%), the method suffered from the impact of matrix effects. A matrix effect percentage of 321.43% was calculated for the analysed samples, with a standard deviation of 6.33%. High matrix effects indicate interaction between the organic material within environmental waters and the compounds of interest, impeding accurate detection. Thus, further optimization is required to minimize these effects.

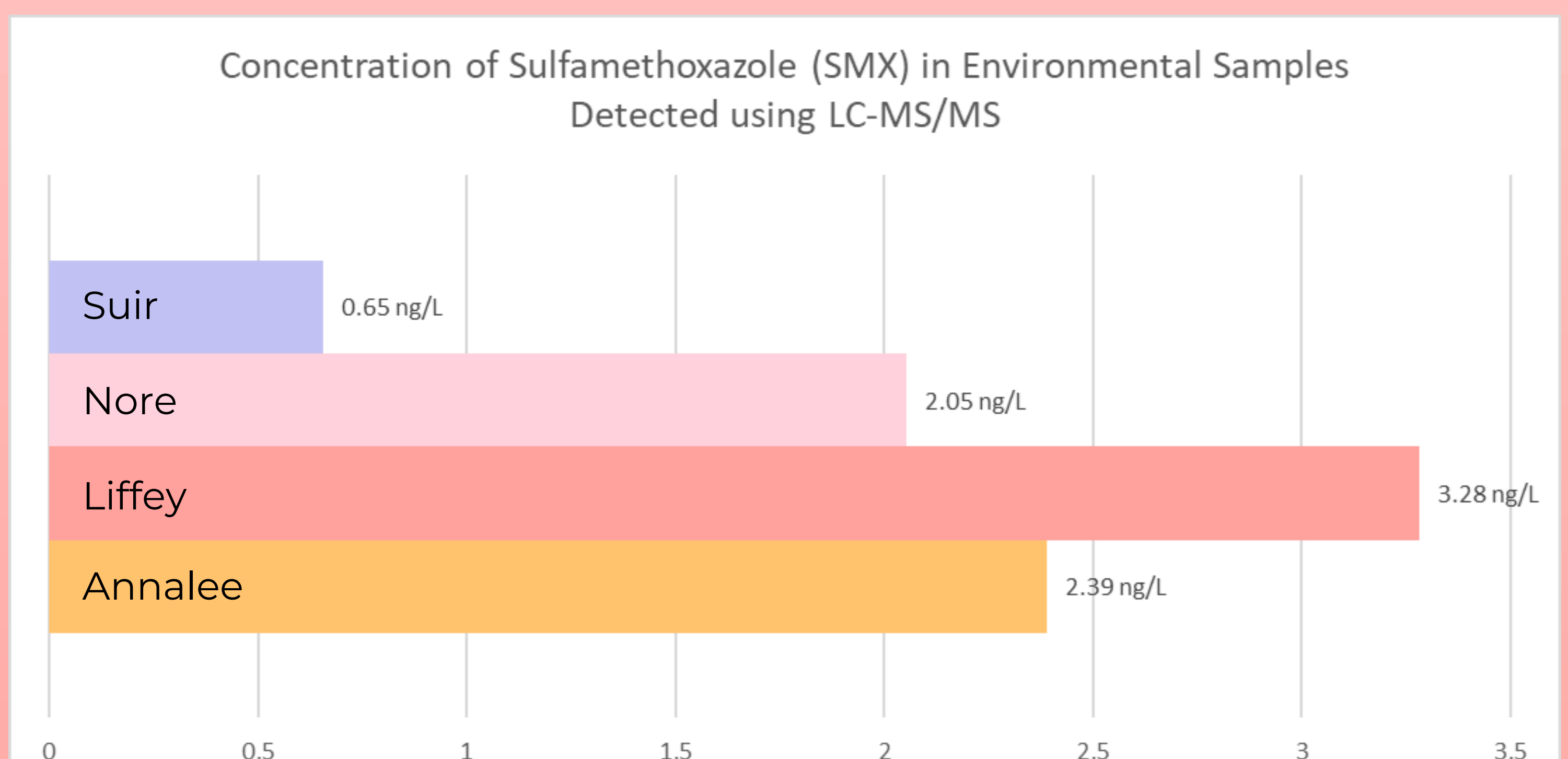


Figure 2 Comparison of Sulfamethoxazole (SMX) levels in Irish surface waters. Samples were obtained from the Rivers Suir, Nore, Liffey and Annalee and analysed using SPE and LC-MS/MS.

Conclusion

A number of factors must be considered for the optimal detection of antibiotics in environmental samples. Firstly, the sample preparation method must be suitable, and allow for a sufficiently large reconcentration factor in order for the instrument to detect the analyte. Secondly, the analytical method itself should be designed taking into account the location and state of the environment that is sampled, i.e., the possibility of analyte degradation and fragmentation.