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Article

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Abstract : Antibiotics have been used to control the aquatic environment in both therapeutic and prophylactic purposes. The issue of antibiotic residues in aquaculture products has aroused much concern over the last decade. The antibiotic residues can remain in food and enter the human body through the food chain, posing great risks to public health. For the safety of foods and products, many countries have issued maximum residue limits and banned lists for antibiotics in aquaculture products. Liquid chromatography tandem mass spectrometry (LC/MS/MS) has been widely used for the determination of trace antibiotic residues due to its high sensitivity, selectivity and throughput. Antibiotics are particularly difficult to extract due to their strong interactions with biological matrices. In this study, LC-MS method was developed and validated for quantitative confirmatory analysis of multi-class antibiotics residues in Shrimp gut, pond water and soil. Nine antibiotics belonging to three wide classes were determined within one chromatographic run.

Keywords: Therapeutic; antibiotic residues; liquid chromatography tandem mass spectrometry (LC/MS/MS); sample pre-treatment; matrix effects .

1.Introduction

Shrimp farming has grown significantly over recent decades, production now exceeds the global catch of all marine shrimps [1].In 2010, the value of shrimp production accounted for approximately 15% of the total value of globally traded fishery products which made shrimps the largest single commodity. In terms of economic value, shrimp markets continue to show positive growth [2]. However, shrimp farming has faced serious disease outbreaks from viral, bacterial and parasitic infections causing significant economic loss[3]. For this reason, antimicrobial drugs have been widely employed to prevent and treat infectious disease. The use of these agents in aquatic animal production has been an issue in the past, and is of increasing concern for consumers in many countries. Aquaculture accounts for nearly half of the world's food fish production, while in South Asia, its contribution exceeds 70% [4].The development of intensive aquaculture in recent decades has resulted in an increase in the occurrence of infectious diseases and mortality of farmed fish and shrimp [5,6]. This led to the extensive use of antibiotics, notably tetracyclines, sulphonamides and quinolones, and, consequently, to their residues in aquaculture products [7]. As a result, different regulators, such as the European Commission, Food and Drug Administration (FDA), Environmental Protection Agency (EPA), [8] and government ministries of various countries established the maximum residual limits (MRLs) of antibiotics in food of animal origin [9,10]. However, such regulations are still absent in most developing countries [11].To meet growing demand, shrimps and other seafood are often cultivated by aquafarms, where many animals are kept in relatively small spaces, making them more prone to diseases[12]. In order to preserve animal health as well as to ensure production and to increase yields, antibiotics are used on a large scale[13].

Residues of these antibiotics in foods of animal origin are a major concern because they are harmful to the consumer's health and could induce pathogens to develop resistance[14].The misuse of these compounds including extra label and inappropriate use, can promote the persistence of drugs in animal products[15].Veterinary drugs are used in animal husbandry and aquaculture for therapeutic or disease-preventive reasons and, in some cases, to promote growth of livestock[16]. Despite when the specified withdrawal periods are not observed, unsafe antibiotic residues, or their metabolites, may be present in edible products such as milk, eggs, and meat [17]. With regard to human health risks, antibiotic residues induce and accelerate antibiotic resistance development, promote the transfer of antibiotic-resistant bacteria to humans, cause allergies (penicillin),and induce other severe pathologies, such as cancers (sulfamethazine, oxytetracycline, and furazolidone), anaphylactic shock, nephropathy (gentamicin), bone marrow toxicity, mutagenic effects, and reproductive disorders (chloramphenicol). Antibiotic resistance, which has excessively increased over the years, is one of the adverse consequences of this phenomenon, constituting a severe public health issue, thus requiring the regulation of antibiotics in all areas, including animal breeding. In order to preserve animal health as well as to ensure production and to increase yields, antibiotics are used on a large scale[18]. Residues of these antibiotics in foods of animal origin are a major concern because they are harmful to the consumer's health and could induce pathogens to develop resistance[19]. The aim of this study was to provide the first, to the best of our knowledge, data on multi-class antibiotic residues in aquaculture shrimp farms in Southern coastal areas of Kerala. For this purpose, a rapid, sensitive and selective method for the simultaneous UPLC-MS/MS determination of 9 antibiotic residues from three different classes in farmed Shrimp samples was developed and validated. There was no specific external funding was received for this study.

2. Materials and Methodology

2.1. Sample collection

Shrimp samples (*Penaeus monodon*) were collected from Southern coastal area farms of Kerala like Munroe Island, Neyyatinkara and Anjuthengu. The control samples, were the larval stages of the shrimp *Penaeus monodon* from these three different farms were maintained in an artificially prepared tank with proper aeration. The shrimp samples (*Penaeus monodon* ,$n = 25$) were collected from Munroe Island, Neyyatinkara and Anjuthengu shrimp farms. Shrimp samples were brought to the laboratory in an ice box from the farms. After removing the shells from shrimp, the samples were blended and stored in a zip bag at −20◦C until further analysis. The respective pond water of 50ml and soil of 25gm were collected from each specific shrimp farms.

2.2.Sample preparation

Both the shrimp samples and shrimp controls were subjected to perform the LC MS analysis separately. The head, shell, tail and intestinal organs of the shrimps were removed prior to analysis. Muscle tissues were blended using a Moulinex mixer, and stored at 20 C until analysis. Then, the targeted residues were extracted from shrimp muscle using simple liquid extraction modified from Freitas A. et al [16]. Briefly, the optimized procedure was as follows; 2.0 ± 0.1 g of sample was weighed in a 50-mL polypropylene centrifuge tube and 500 µl of 1% formic acid in water and 7.0 mL of acetonitrile (ACN) were added and the mixture was mixed for 20sec. The mixture was centrifuged approximately at 5000rpm 10 min. The acetonitrile layer (supernatant) was transferred into a 15

mL glass test tube and the extraction procedure were repeated. The combined supernatant (acetonitrile) was evaporated to dryness at 40 °C with nitrogen stream using Turbo vap. The dried extract was reconstituted with 2 mL of n-hexane and 1.0 mL of 0.1% methanol in water. All individual standards stock were prepared in the methanol. Further mixture of all antibiotics were prepared in methanol. This stock was serially diluted to prepare calibration levels ranging from 0.5 ppb to 50 ppb in methanol for solvent standard and in matrix for matrix matched standard calibration. The mixture was transferred to a 15 mL polypropylene centrifuge tube, mixed (10 s) and centrifuged at 10,000rpm (5 min).The bottom layer was transferred and filtered through a 0.2 μm syringe) filter. The collected filtrate was injected into the UPLC-MS/MS system under the optimized analytical conditions. The MS/MS parameters of the selected antibacterial drugs were shown in table 1.

2.3. Instrumental and chromatographic conditions

Instrument Parameters

Instrument Used is Agilent 646460QQQ Mass spectrometry detector coupled with Agilent 1290 Infinity series UHPLC system.

HPLC Conditions

Column	: RP C18 (150 x 4.6mm) 5μm
Mobile phase	: A. 0.1% Formic Acid in water
	B. Acetonitrile
Flow rate	: 0.4ml/min
Injection volume	: 20μl
Detector	: Mass spectrometer

Mass spectrometer Conditions

Ionisation	: ESI
Polarity	: Positive
Scan type	: MRM

Serial Number	Compound Name	Precursor Ion	Product Ion	Polarity
1	Ciprofloxacin RPT	332	314.1	Positive
			231.1	Positive
2	Danofloxacin	358.1	340.1	Positive
			255	Positive
3	Difloxacin	400.1	382.2	Positive
			356.2	Positive
4	Doxycycline	445	154	Positive
			428	Positive
5	Enrofloxacin RPT	360	342.2	Positive
			70.1	Positive
6	Flumequin RPT	262	126.1	Positive
			99.1	Positive
7	Levofloxacin RPT	362	318.2	Positive
			75.1	Positive
8	Lomefloxacin RPT	352	99.1	Positive
			75.1	Positive
9	Nalidixic acid RPT	233	215.1	Positive
			104.1	Positive
10	Norfloxacin RPT	320	302.1	Positive
			189.1	Positive
11	Ofloxacin RPT	362.1	318.1	Positive
			261.1	Positive
12	Oxolinic acid RPT	262	244.1	Positive
			75.1	Positive
13	Oxytetracycline	461	443.2	Positive
			426.2	Positive
14	Sarafloxacin RPT	386	368.1	Positive
			255.1	Positive
15	Sulfa Chloropyridazine	285	156	Positive
			65.1	Positive
16	Sulfa Diazine	251	156	Positive
			80.1	Positive
17	Sulfa Dimethoxine	311.1	218	Positive
			156.1	Positive
18	Sulfa Dimidin	279	186	Positive
			80.1	Positive

Table 1: The MS/MS parameters of the selected antibacterial drugs

Serial Number	Compound Name	Precursor Ion	Product Ion	Polarity
19	Sulfa Doxin	311.1	156	Positive
			108	Positive
20	Sulfa Merazin	265	156	Positive
			80.1	Positive
21	Sulfa Methizole	271	156	Positive
			65.1	Positive
22	Sulfa Methoxazole	254	155.9	Positive
			80.2	Positive
23	Sulfa Methoxypyridazine	281	156	Positive
			80.1	Positive
24	Sulfa pyridine	250	184.1	Positive
			156.1	Positive
25	Sulfa Thiazole	256	156	Positive
			80.1	Positive
26	Tetracyline	445.1	427.2	Positive
			410.2	Positive
27	Trimethoprim	291	81.1	Positive
			51.1	Positive

Table 1: (Continued)

2.4 Method application

The developed method was applied to real samples that were collected from different shrimp farms in the southern coastal areas of Kerala .A matrix-matched calibration, a reagent blank, and a spiked blank sample were used for quality control. Furthermore, following the criteria defined by Commission Decision 2002/657/EC, the retention time and the ion ratio of the detected antibiotic in a real sample were compared to those of matching calibration standards in the same batch to ensure the identity of the detected molecule. Twenty-five samples were analysed for simultaneous detection using LC-MS/MS analysed.

2.5 Chemical and standards

Twenty three commonly detected antibiotics that belong to three antibiotics classes like Trimethoprim, Quinolones & Fluoroquinolones and Sulfonamides respectively were selected for specific antibiotic residue analysis.12quinolones/fluroquinolons(OXA,NAL,LFLX,Sarafloxacin,FLM/FLU, CIN, Danofloxacin,DIF,OFX,NOR,ENRO ,CIP,10sulfonamides(SDZ,STZ,SMR/SMZ,SMZ/SMT,SMT/SMZ/SDI,SMP/STP,SCP,TMPSMX,TMPSMZ/SXT,SDM,SP and Trimethoprim. All the above antibiotic standards were prepared by dissolution in methanol and kept at −20°C, and all solvents were of HPLC grade. Ultra-pure water was used in this study (Milli-Q ultrapure water system, Millipore, Bedford, MA, USA).

3.Results and Discussion

3.1Antibiotic residues

Twenty-three antibiotics belonging to three classes were analysed in Southern coastal area shrimp farms including 12 Quinolones/Fluroquinolones(OxolinicAcid,NalidixicAcid,Lomefloxacin, Sarafloxacin,Flumequin,Cinoxacin,Danofloxacin,Difloxacin,Ofloxacin, Norfloxacin, Enrofloxacin ,Ciprofloxacin)10 Sulfonamides (Sulfadiazin, Sulfathiazole,Sulfamerazin,Sulfamethizole,Sulfadimidin/Sulfamethazin, Sulfamethoxypyridazin,Sulfadoxin,Sulfachloropyridazin,Sulfamethoxazole,Sulfadimethoxine and Trimethoprim.

The concentration of detected antibiotics ranged from < LOQ to 9.58 × 105 ng/L. Quinolones had the highest detection frequencies (83%) followed by sulfonamides (72%) and (69%) trimethoprim. The samples shrimp gut, soil and pond water obtained from the southern coastal areas of Kerala, Munroe Island shrimp farm were clearly observed with the presence of antibiotic residues in LC MS analysis. The shrimp gut is observed with the residues like sulfamethoxazole and norfloxacin antibiotics. The pond water were observed with the residues like norfloxacin and sulfamethoxazole antibiotics. The soil sediment were observed with the residues like sulfamethoxazole and norfloxacin antibiotics.

Antibiotics have an elimination time where, at a certain period, the residual content is no longer detected in body tissues (Avunje et al. 2021). Each antibiotic has a different elimination time. The acquisition time of norfloxacin residue of samples in LC MS analysis is 4.37min . The acquisition time of sulfamethoxazole residue of samples in LC MS analysis is 7.03 min. The samples shrimp gut, soil and pond water obtained from the southern coastal area of Kerala, Anjuthengu shrimp farm showed the presence of antibiotic residues in LC MS analysis. The pond water were observed with the residues like ciprofloxacin and sulfadimidine antibiotics. The soil sediment were observed with the residues like sulfadimidin and ciprofloxacin antibiotics. The shrimp gut were observed with the residues like ciprofloxacin and sulfadimidine. The acquisition time of ciprofloxacin residue of samples in LC MS analysis is 4.54min . The acquisition time of sulfadimidin residue of samples in LC MS analysis is 6.14 min. The samples shrimp gut, soil and pond water obtained from the southern coastal area of Kerala, Neyyatinkara shrimp farm showed the presence of antibiotic residues in LC MS analysis. The shrimp gut were observed with the residues like sulfadiazine and trimethoprim. The pond water were

observed with the residues like trimethoprim and sulfadiazin antibiotics. The soil sediment were observed with the residues like trimethoprim and sulfadiazine antibiotics. The acquisition time of sulfadiazin residue of samples in LC MS analysis is 4.16 min. The acquisition time of trimethoprim residue of samples in LC MS analysis is 3.24 min. For the quinolones and fluroquinolones compounds tested, Norfloxacin was the most frequently detected residue in shrimp gut samples from Munroe Island(82%) but the concentrations were higher than LOQ and were not detected in Anjuthengu and Neyyatinkara <LOQ – 73 ng/L and <LOQ – 245 ng/L respectively. For sulfonamide compounds tested, sulfamethoxazole were the most frequently detected residue in water samples from Munroe Island(92%).The concentrations were equal to LOQ and were not detected in Anjuthengu and Neyyatinkara <LOQ – 73 ng/L and <LOQ – 245 ng/L respectively.

The simultaneous analysis of the control sample the larval shrimps from same shrimp farms were maintained in artificial tank with natural food supply and water. After attaining the adult stage the shrimp gut, tank water and sediments were analysed with the same parameters and we couldn't find any antibiotic residues in the respective samples. For sulfonamide compounds tested, sulfadimidin were the most frequently detected residue in shrimp gut, pond water and soil samples from Anjuthengu(92%) .The concentrations were equal to LOQ and were not detected in samples from Munroe Island and Neyyatinkara .The trimethoprim residues were absent in shrimp gut, water and soil the samples from Munroe Island and Anjuthengu. Trimethoprim were detected in Shrimp gut,soil and pond water samples from Neyyatinkara The simultaneous analysis of the control samples shrimp gut ,pond water and soil samples were analysed with the same parameters and we couldn't find any antibiotic residues. For sulfonamide compounds tested, Sulfadiazin were present in shrimp gut, pond

water and soil samples from Neyyatinkara. For sulfonamide compounds tested, Sulfadiazin were absent in shrimp gut,pond water and soil samples from Anjuthengu and Munroe Island. The control samples showed no antibiotic residues belonging to quinolons and fluroquinolons, Sulfonamides and trimethoprim.

Analysis was performed using aqueous as well as matrix matched standards. Auto MRM optimization feature was used for optimization for MRM transitions. The matrix matched calibration levels were prepared and injected in segmented MRM mode. The MS /MS analysed chromatogram curve of antibiotic residues of the respective samples were depicted in fig 1-72.The control samples from the respective farms were shown in Figure 73-84.

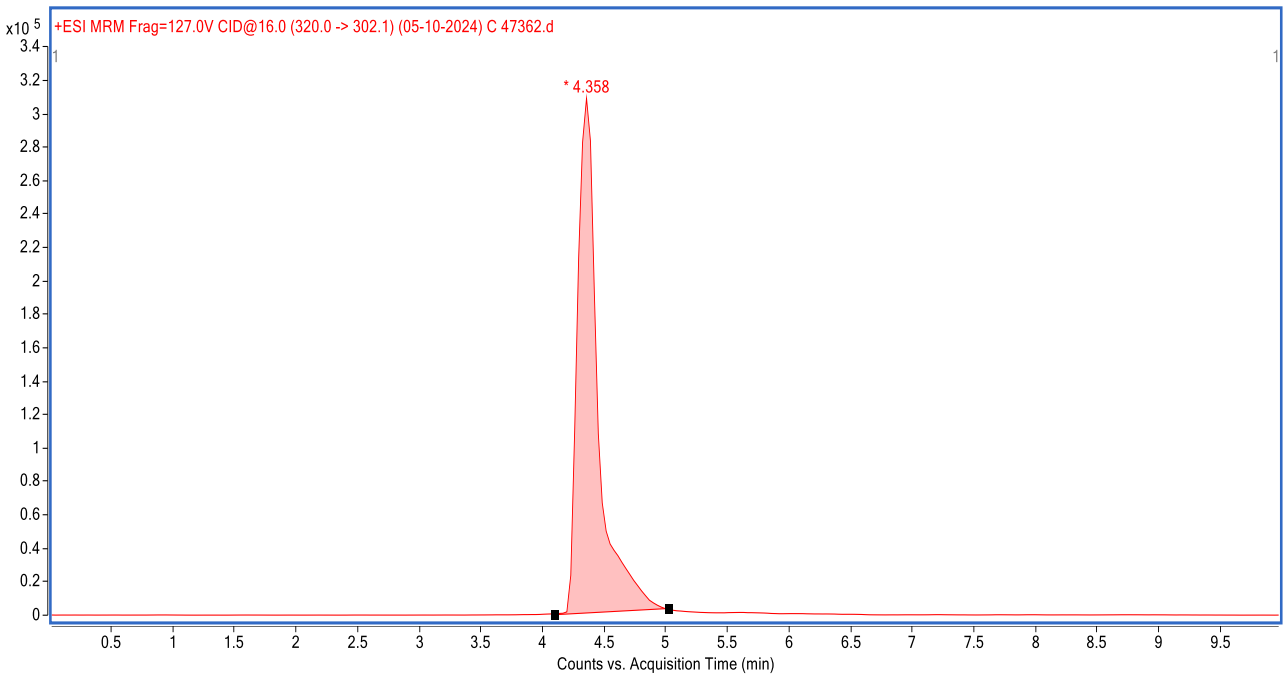


Fig 1:Norfloxacin quantifier ion

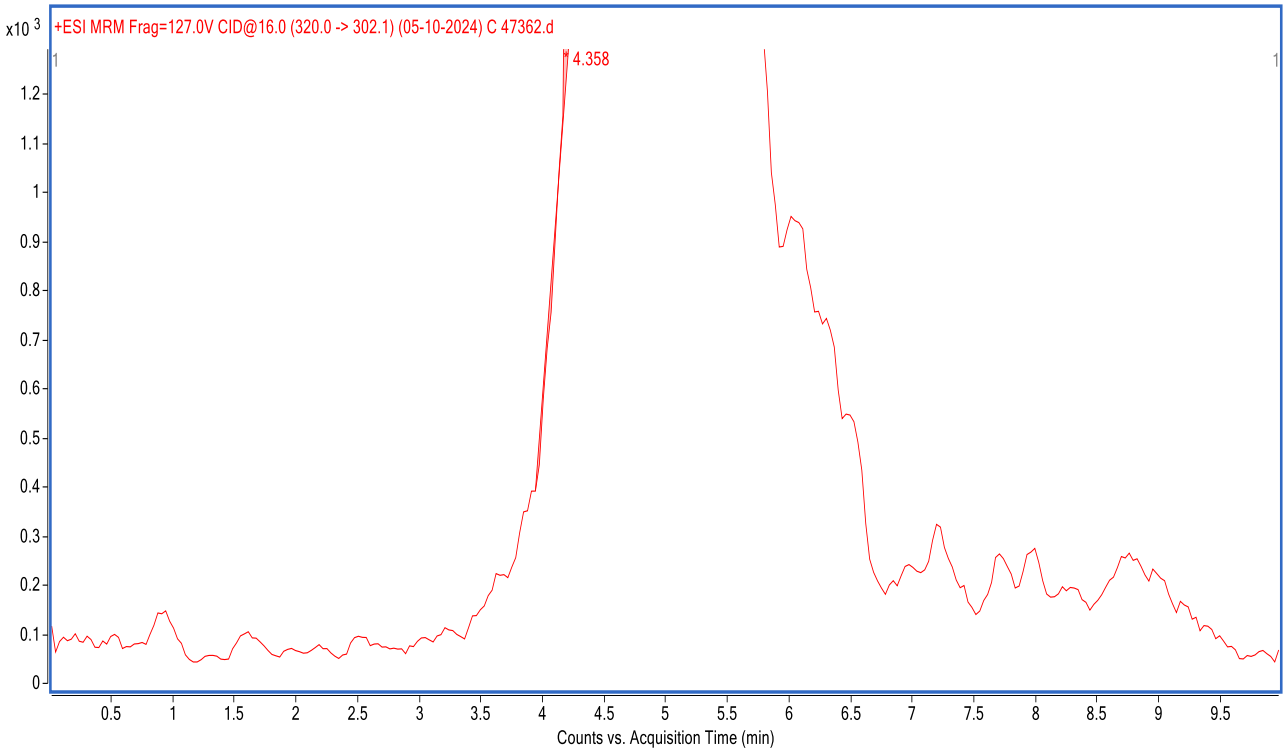


Fig 2:Norfloxacin quantifier ion zoom view

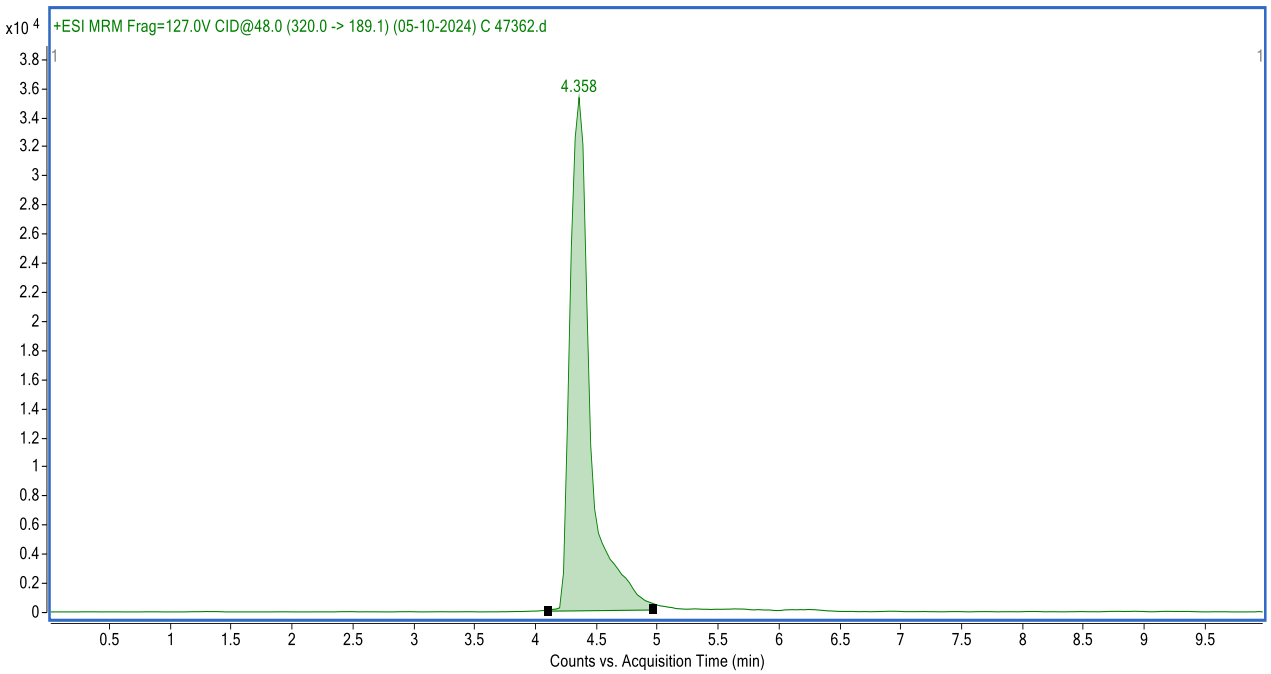


Fig 3:Norfloxacin Qualifier ion

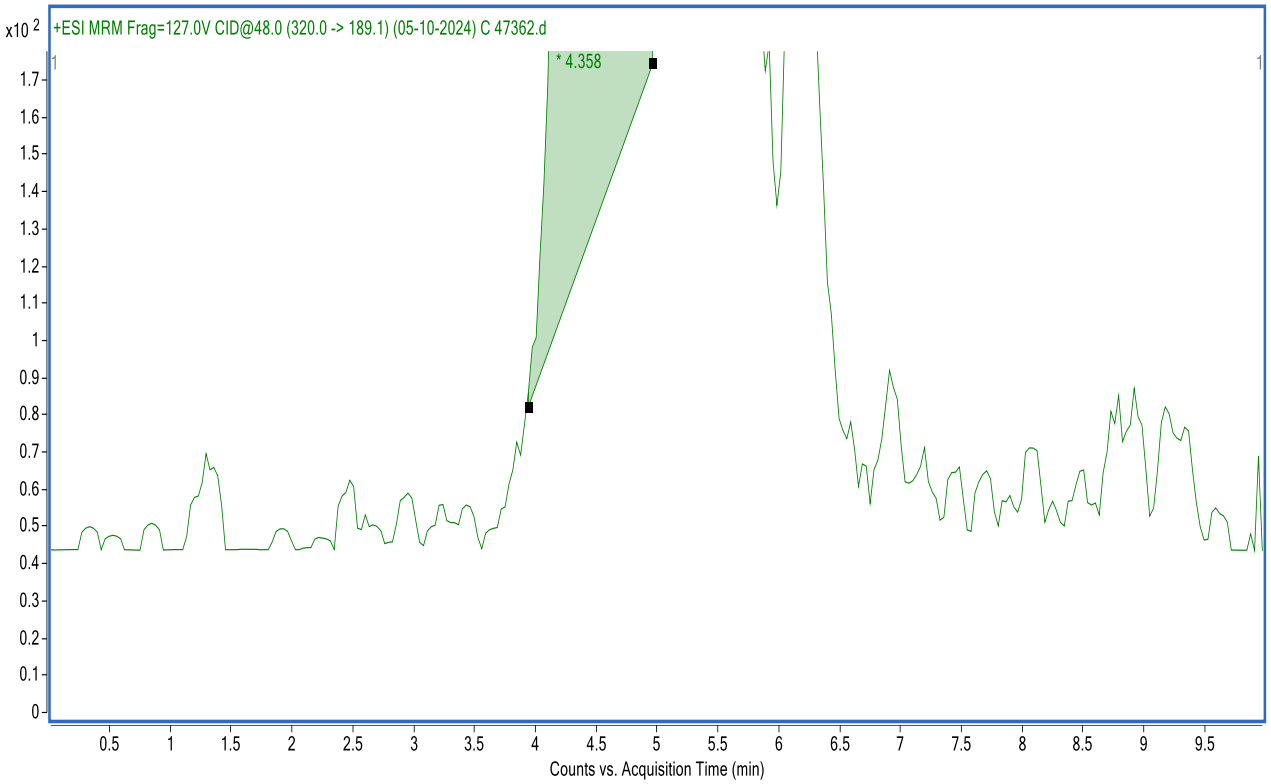


Fig 4:Norfloxacin Qualifier ion Zoomed view

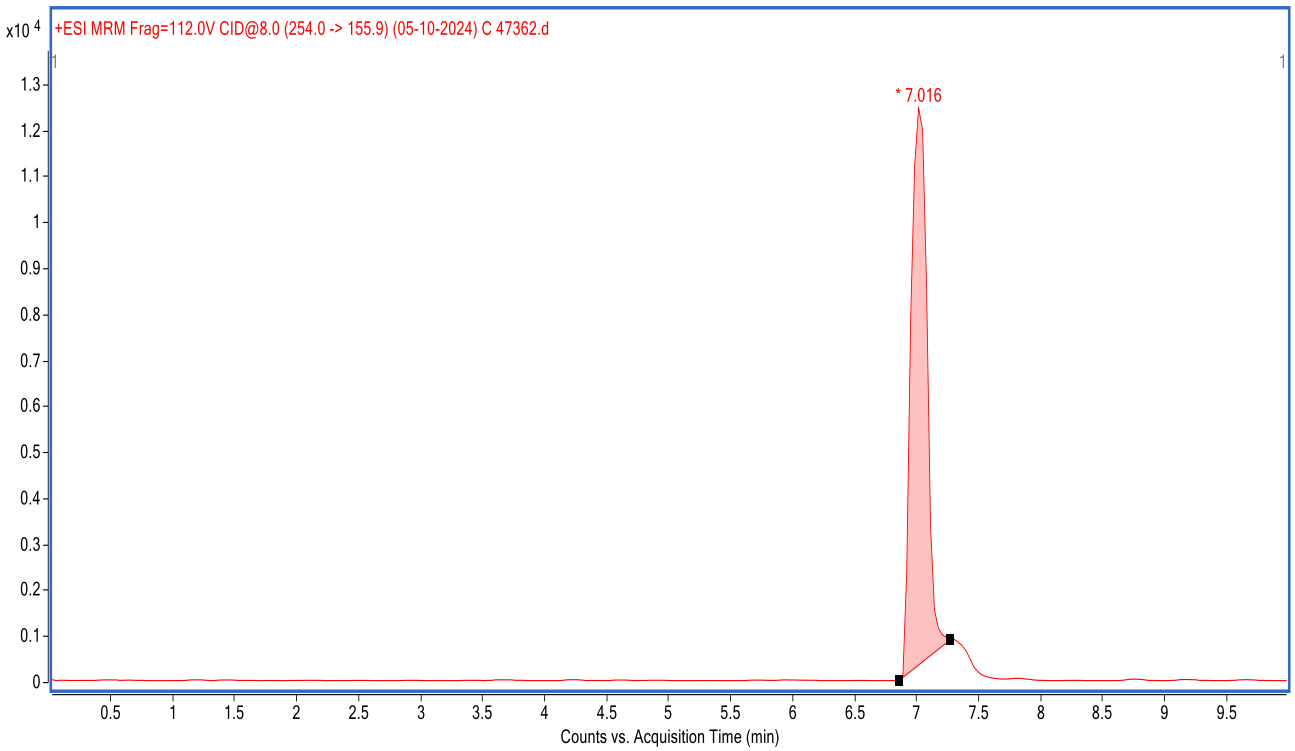


Fig 5:Sulfamethoxazole Quantifier ion

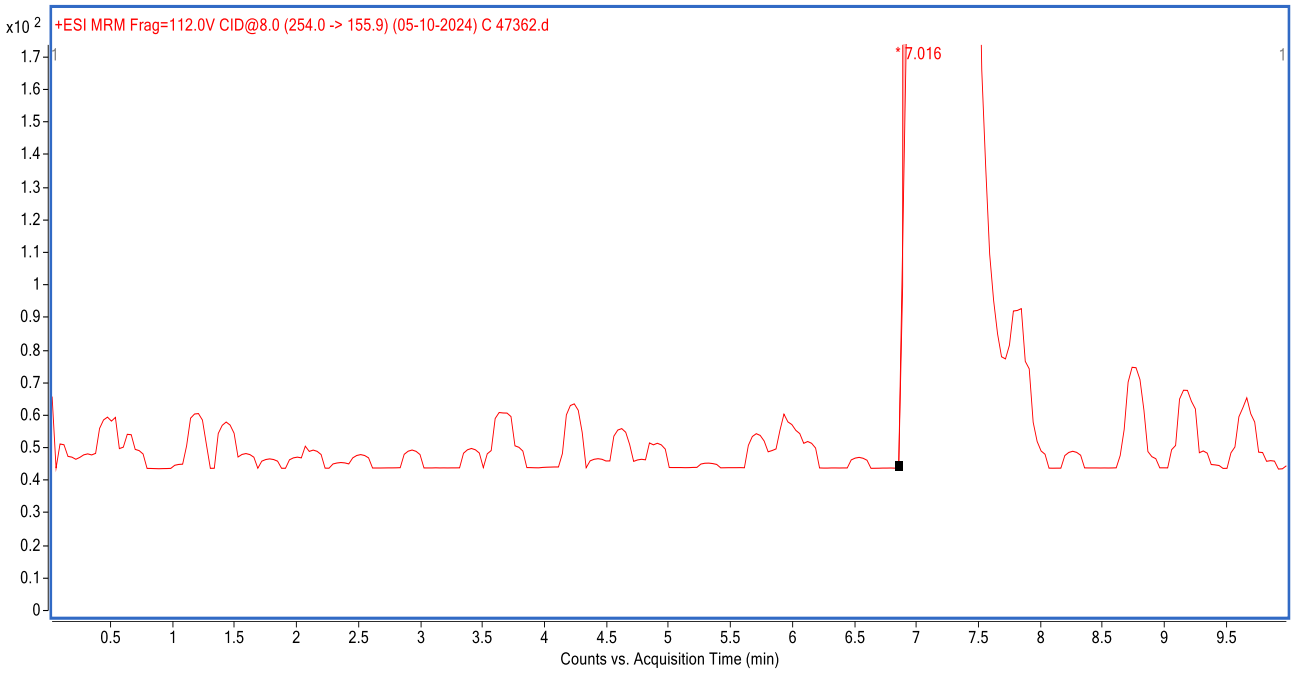


Fig 6:Sulfamethoxazole Quantifier ion Zoomed view

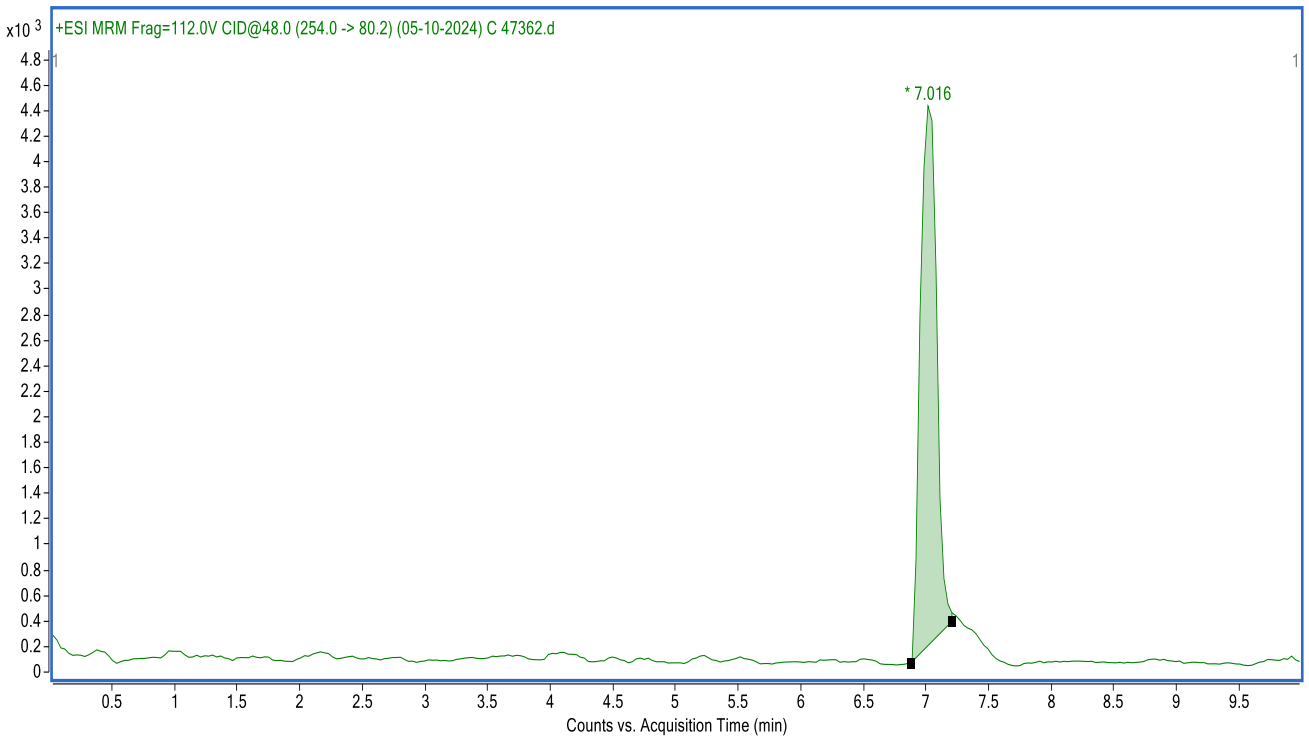


Fig 7:Sulfamethoxazole Qualifier ion

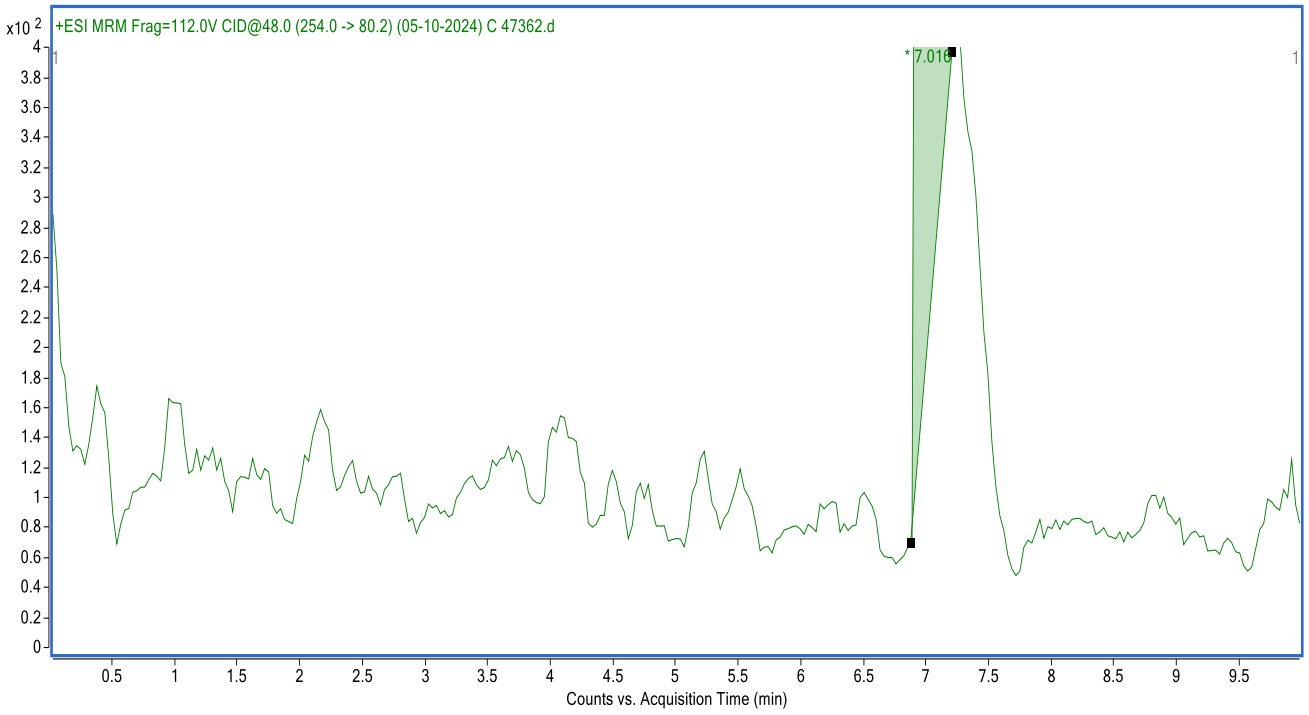


Fig 8:Sulfamethoxazole Qualifier ion Zoomed view

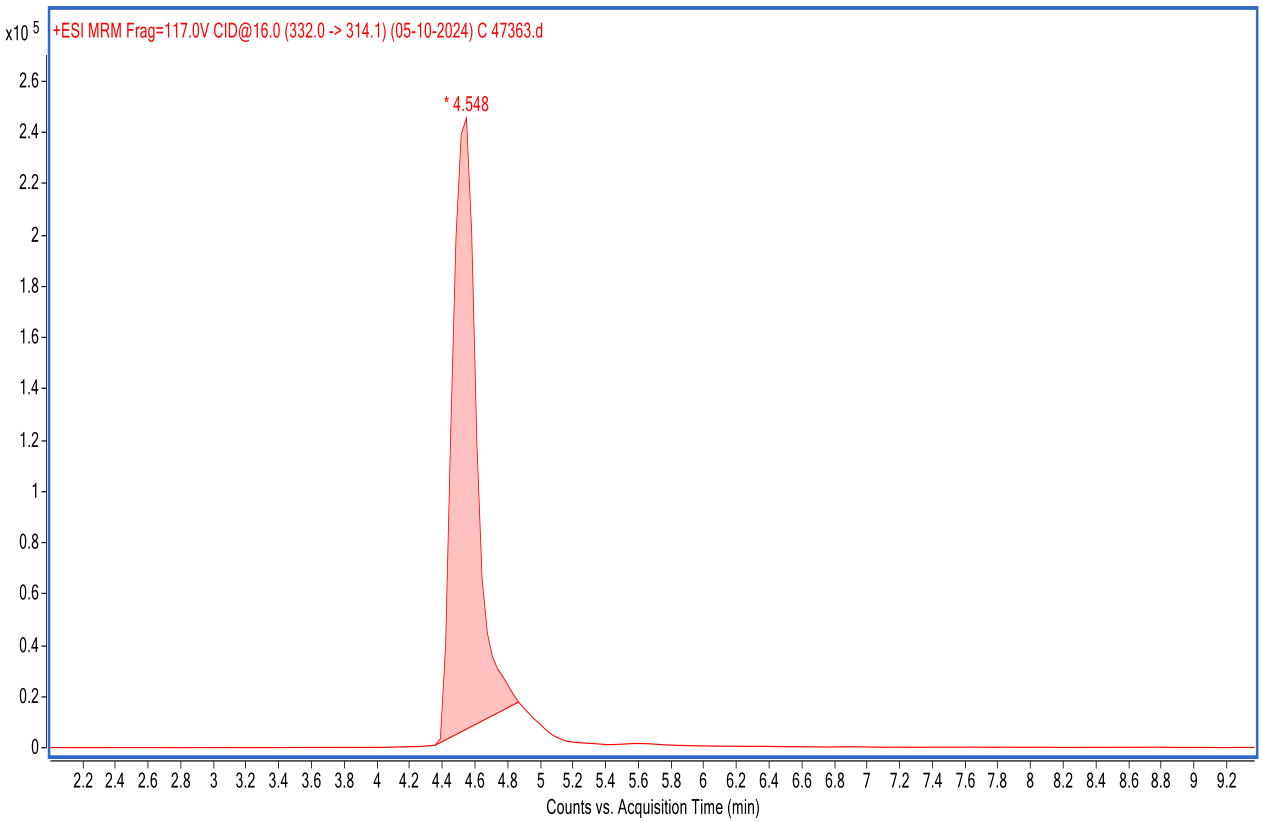


Fig 9: Ciprofloxacin_Quantifier ion

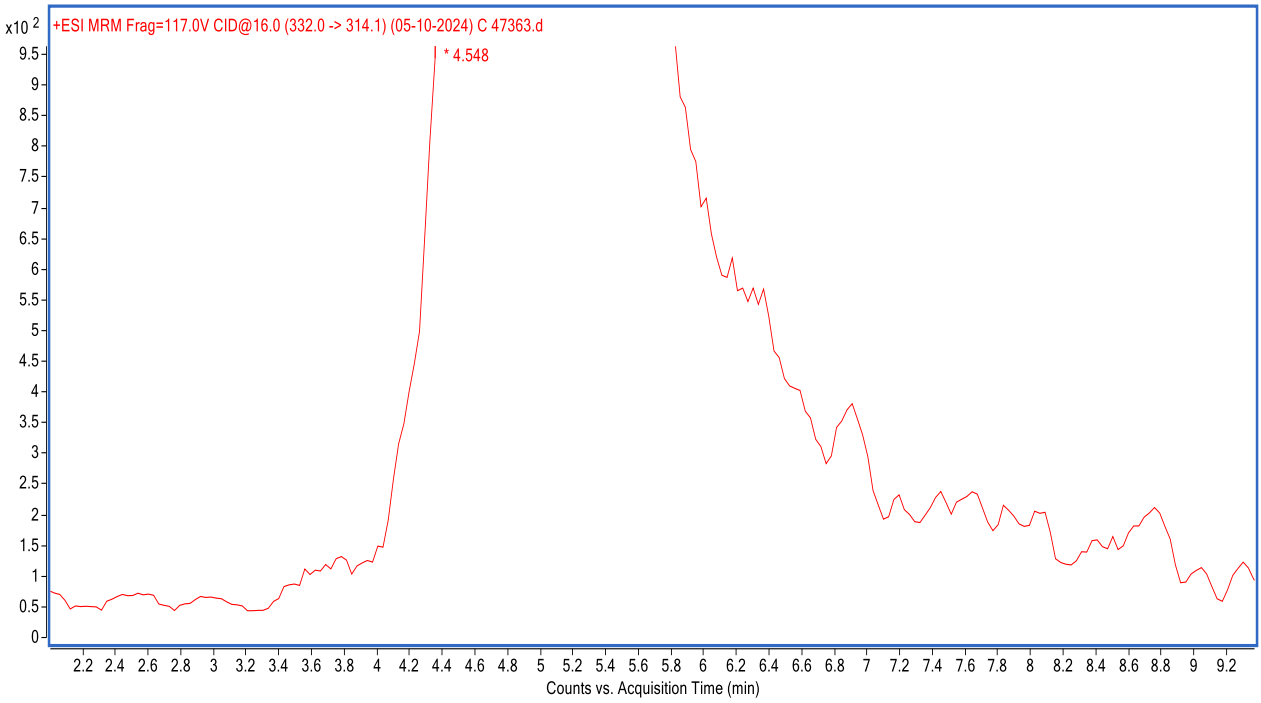


Fig 10: Ciprofloxacin_Quantifier ion Zoomed view

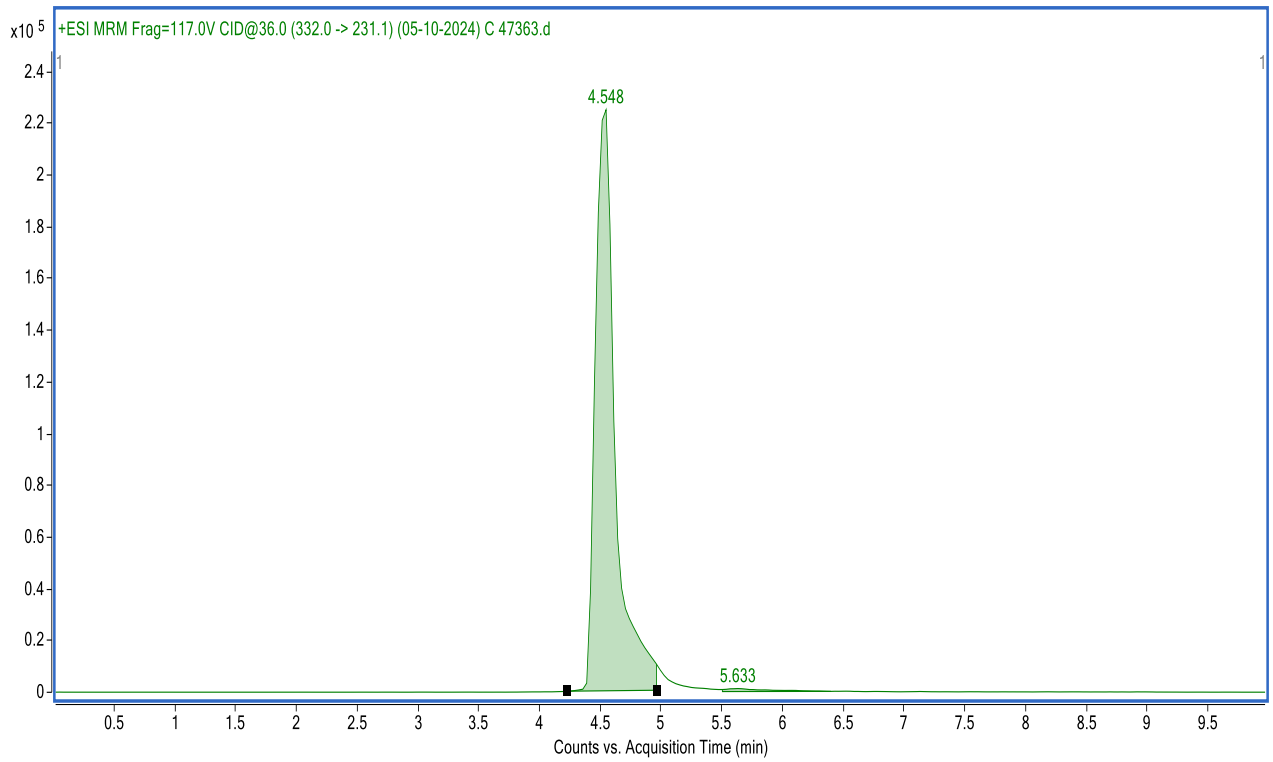


Fig 11: Ciprofloxacin_Qualifier ion

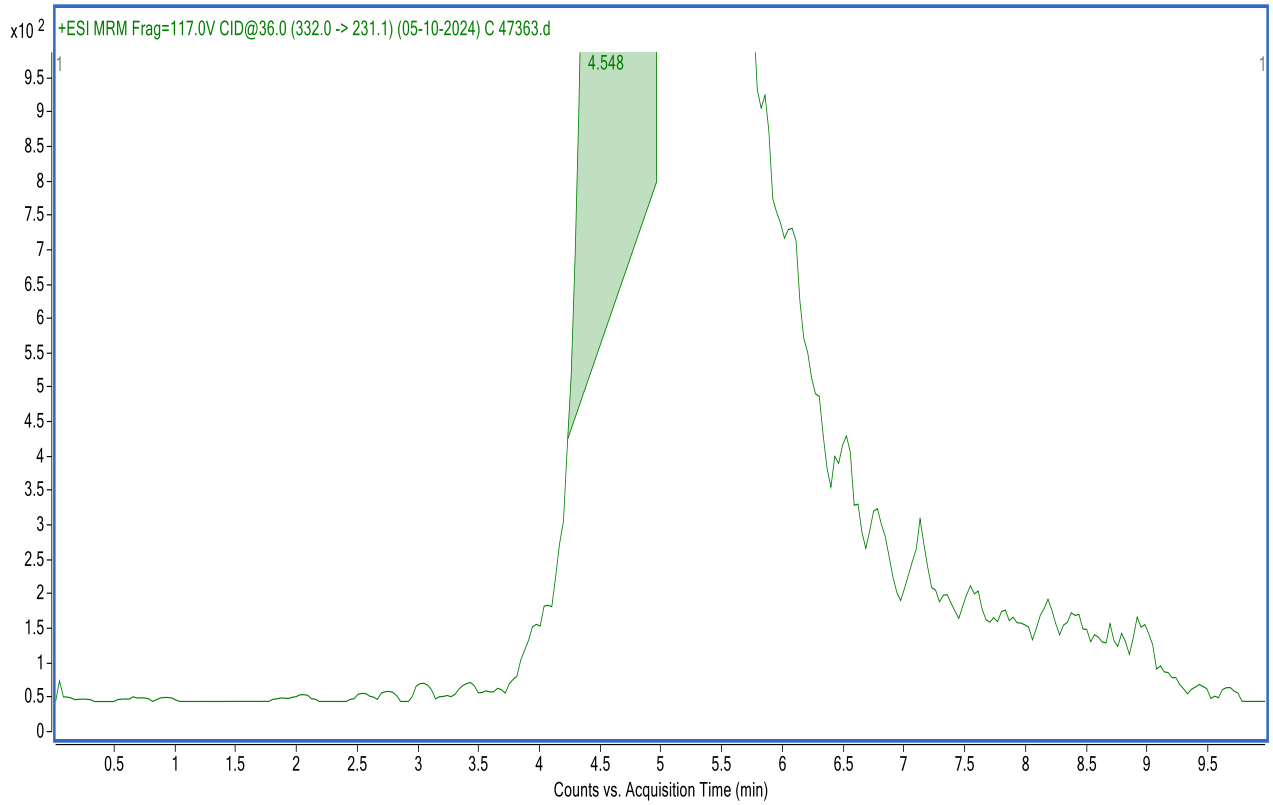


Fig 12: Ciprofloxacin_Qualifier ion Zoomed view

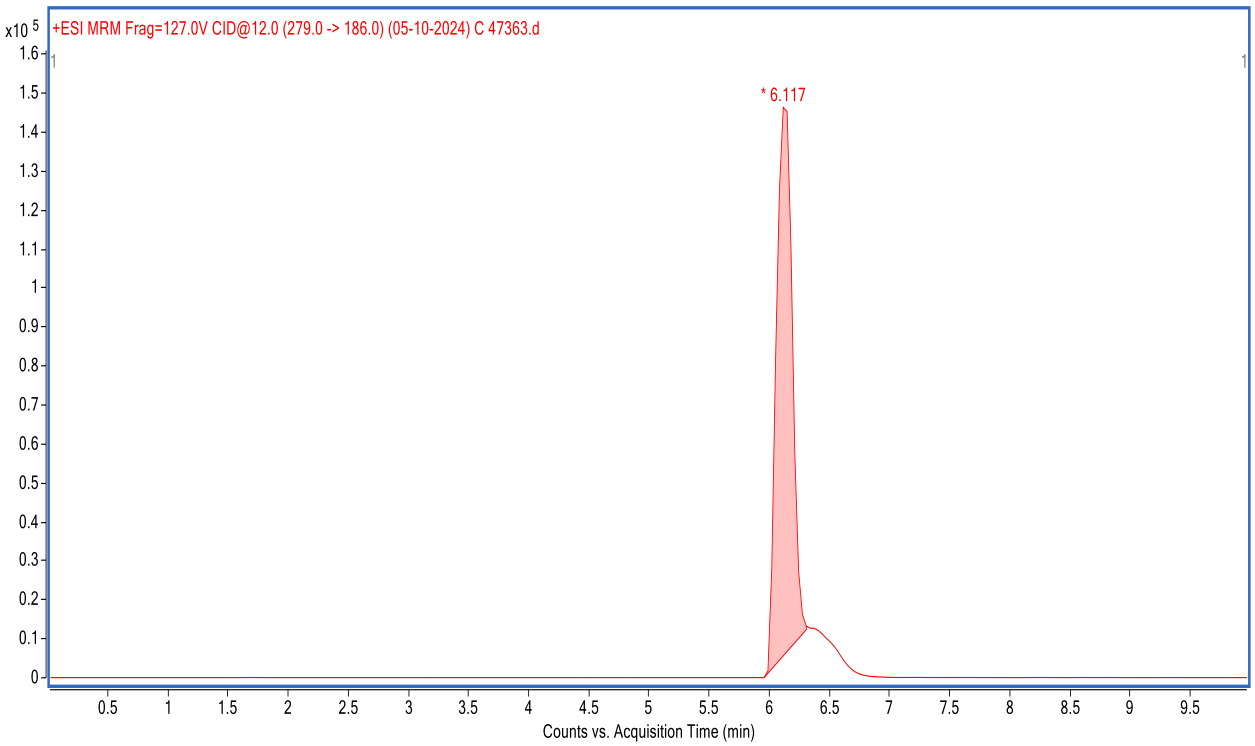


Fig 13: Sulfadimidin Quantifier ion

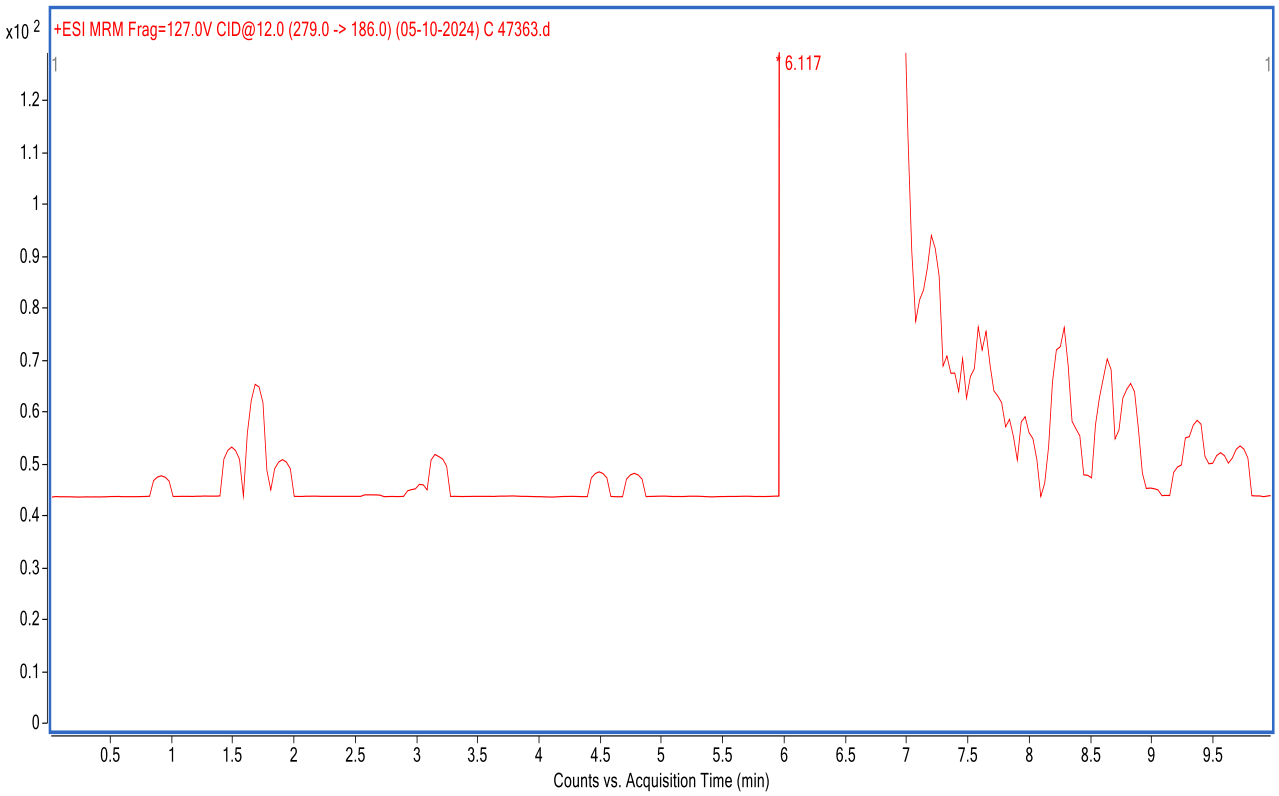


Fig 14: Sulfadimidin Quantifier ion zoomed view

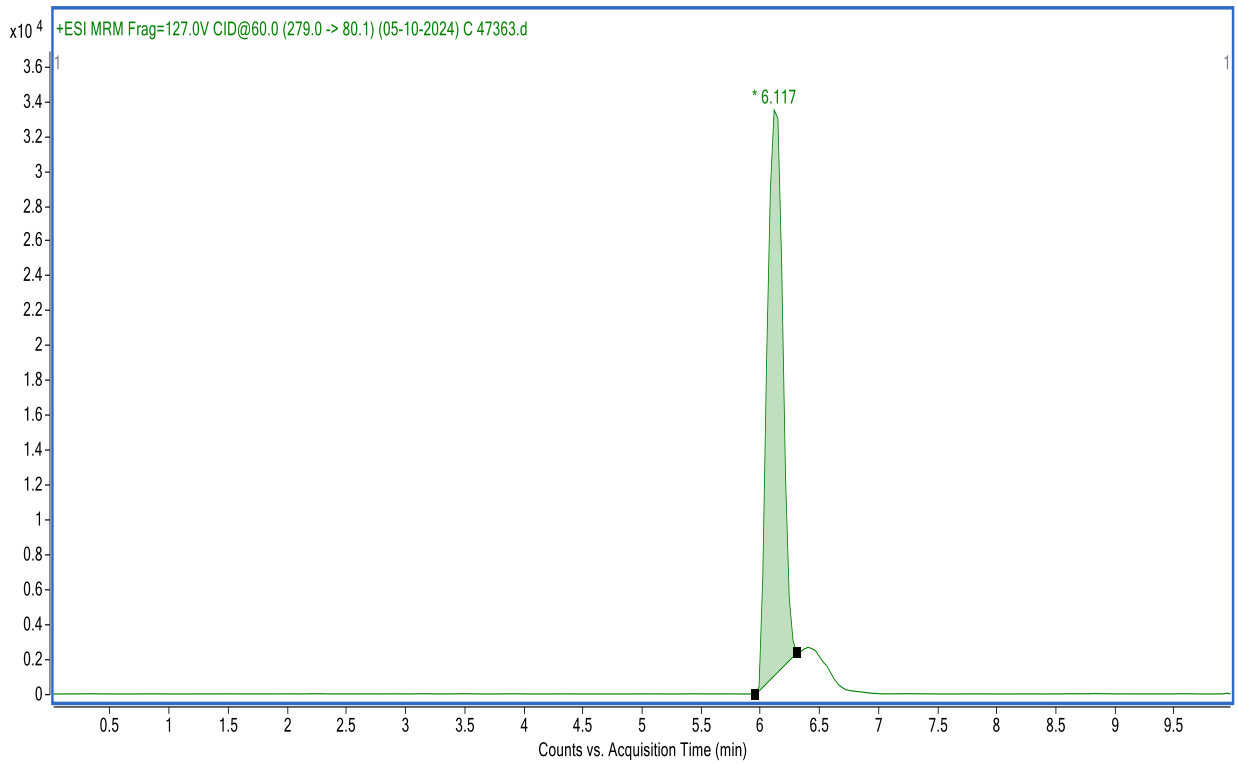


Fig 15: Sulfadimidin Qualifier ion

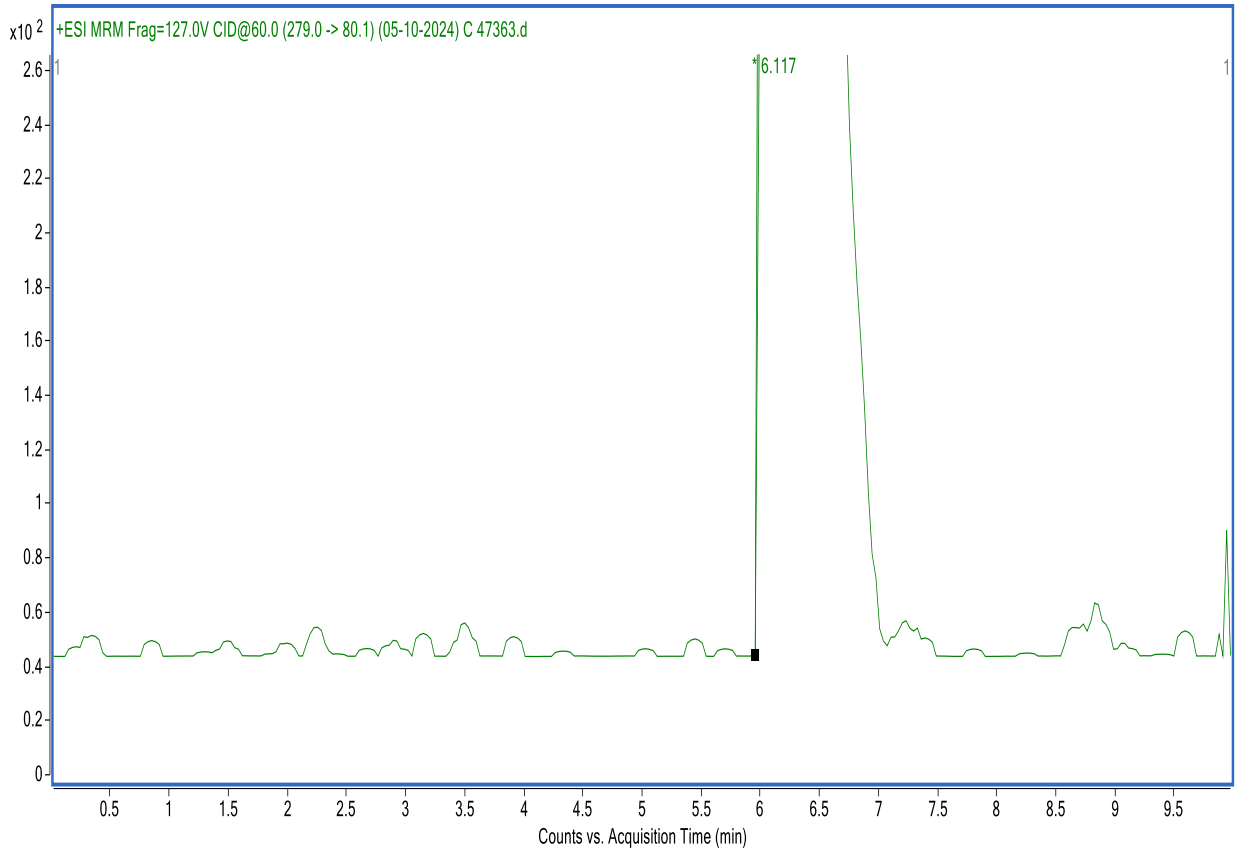


Fig 16: Sulfadimidin Qualifier ion zoomed view

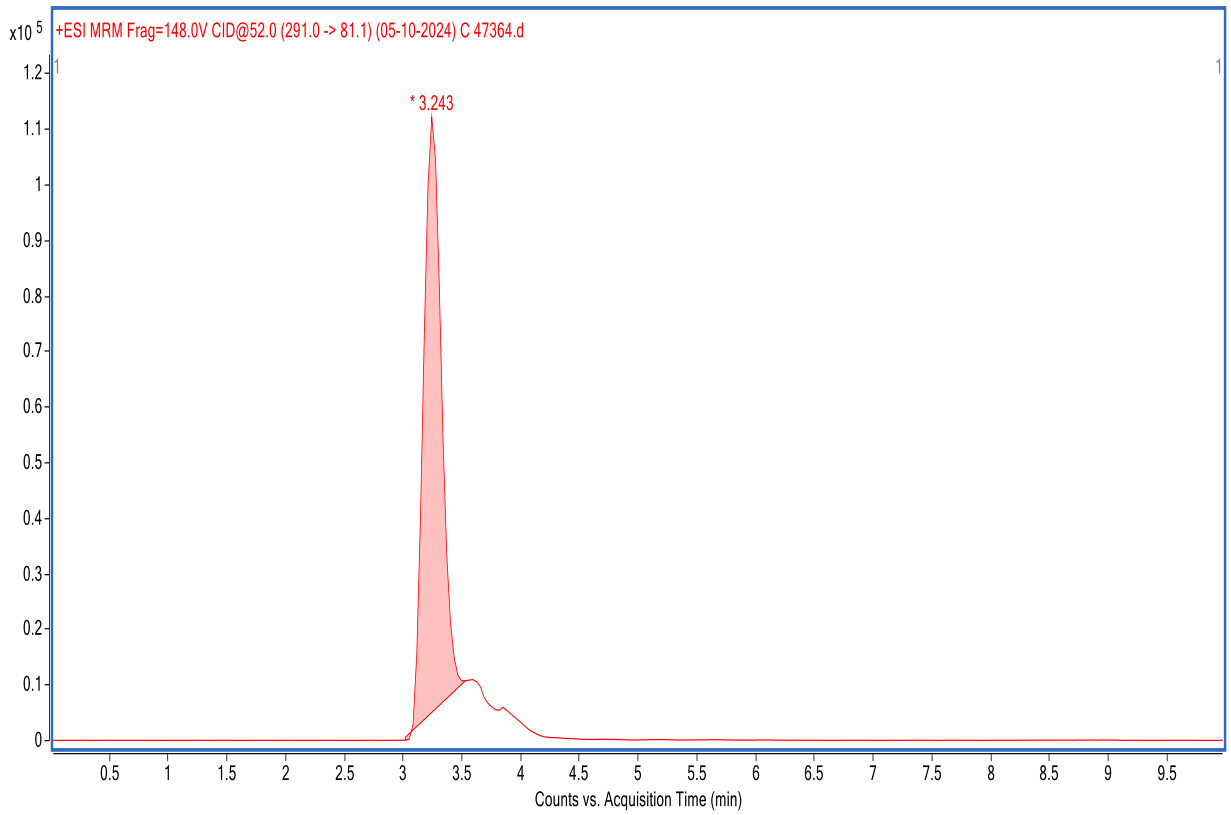


Fig 17: Trimethoprim Quantifier ion

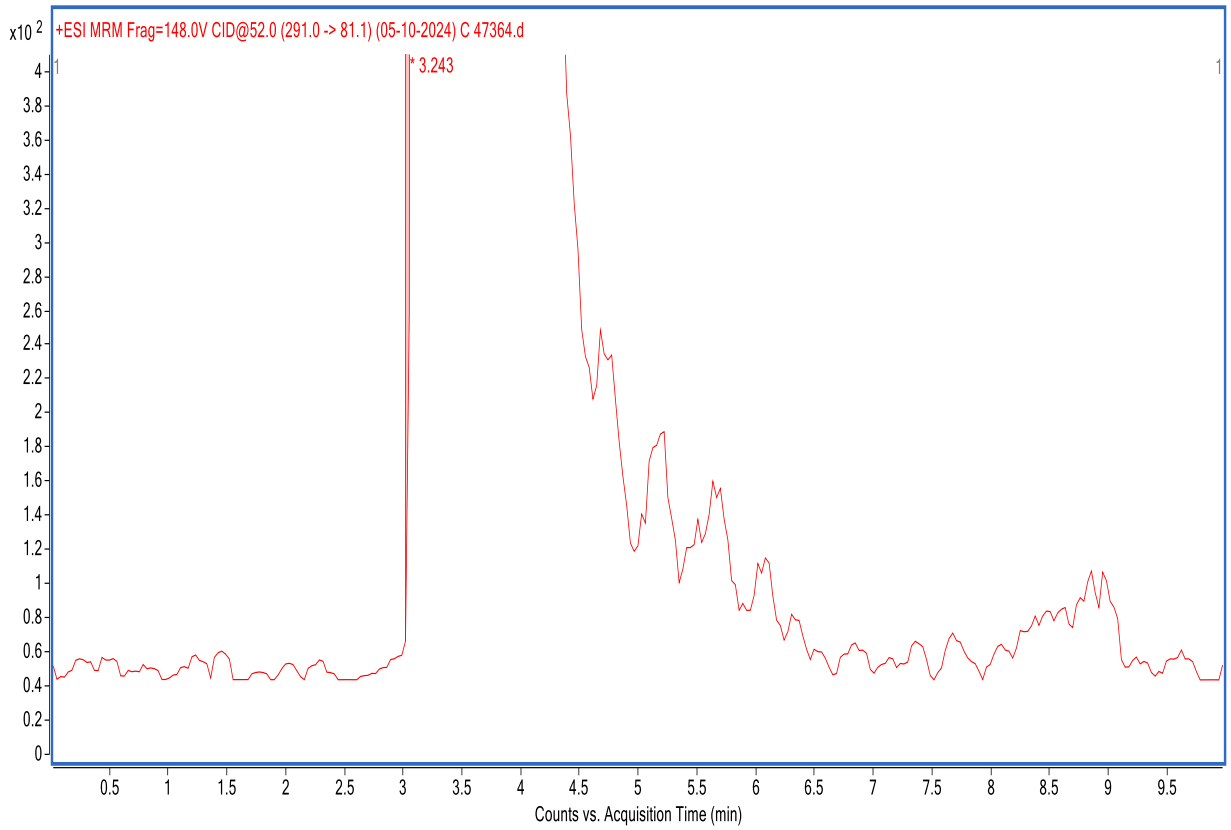


Fig 18: Trimethoprim Quantifier ion zoomed view

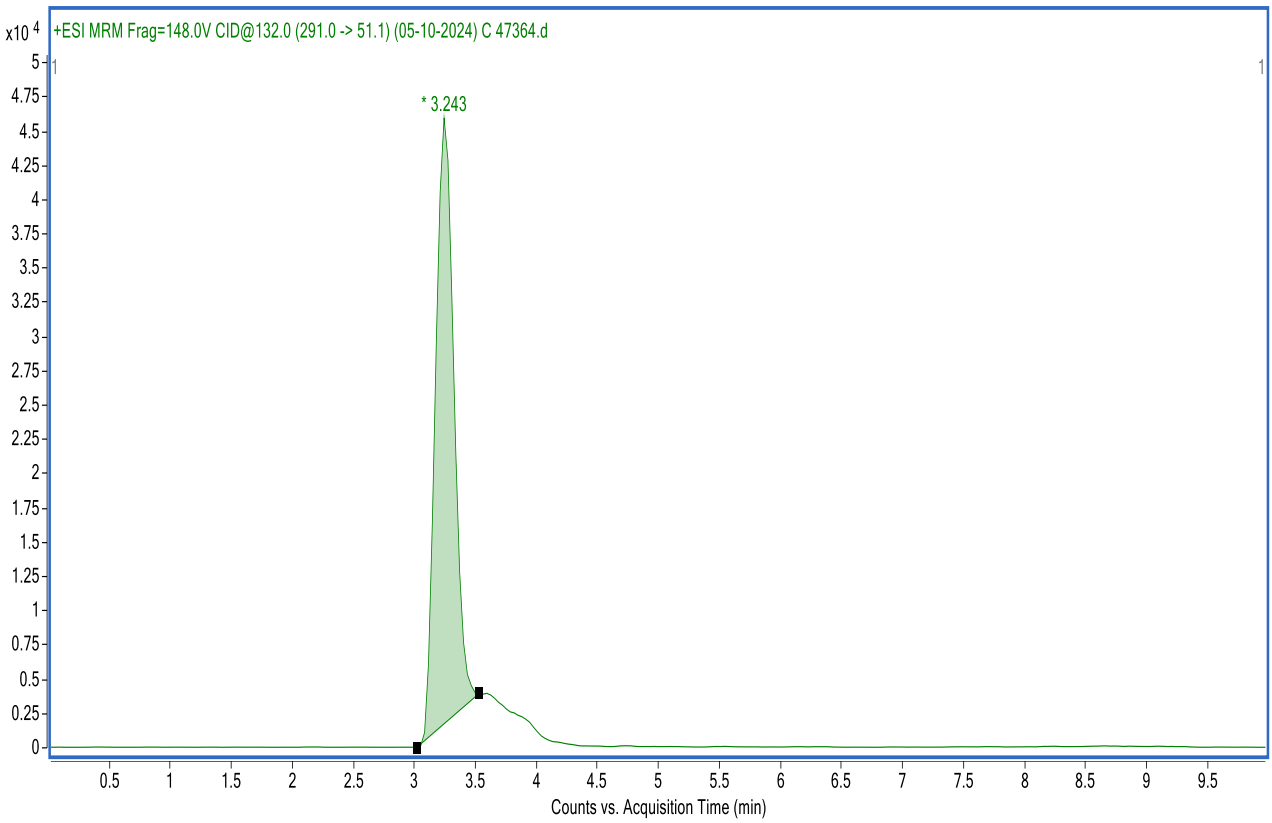


Fig 19: Trimethoprim Qualifier ion

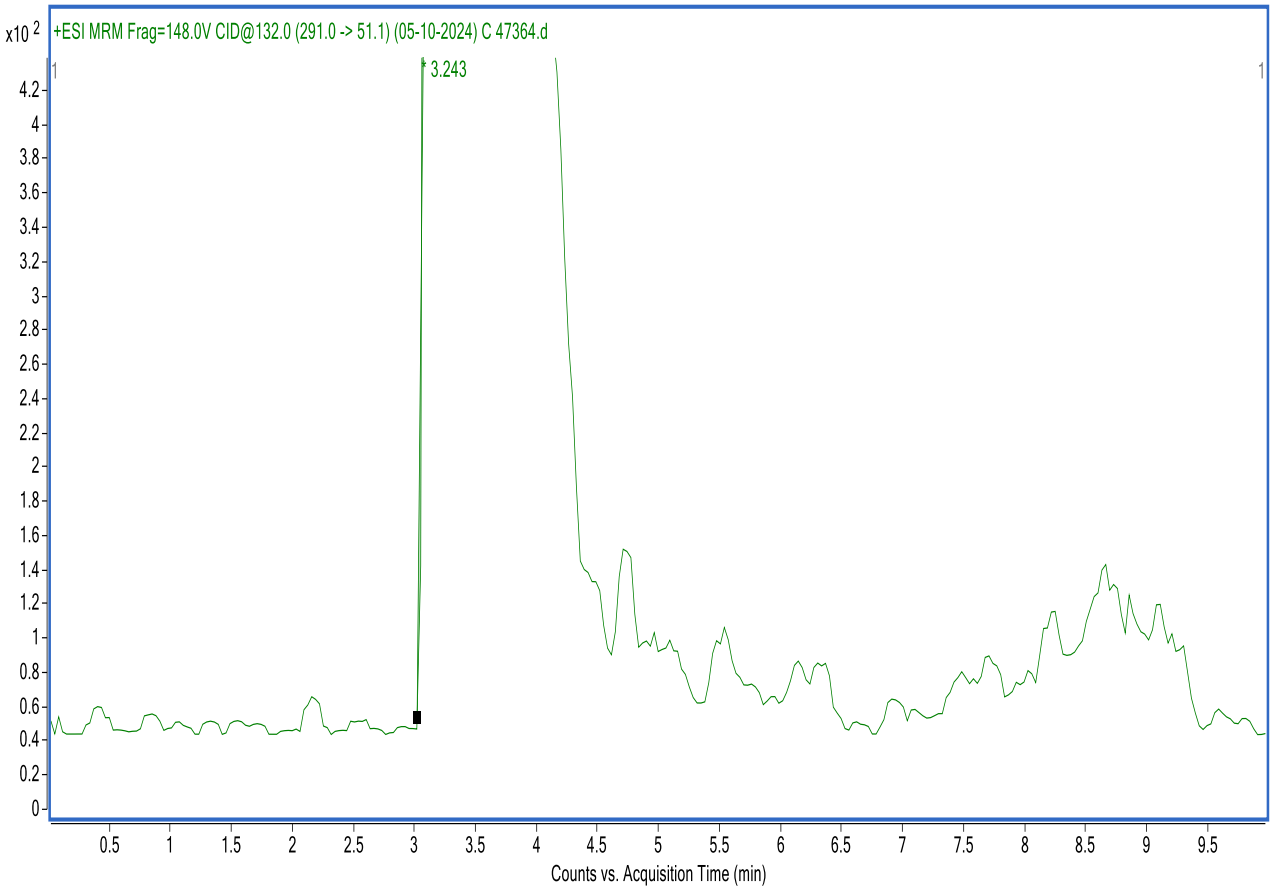


Fig 20: Trimethoprim Qualifier ion zoomed view

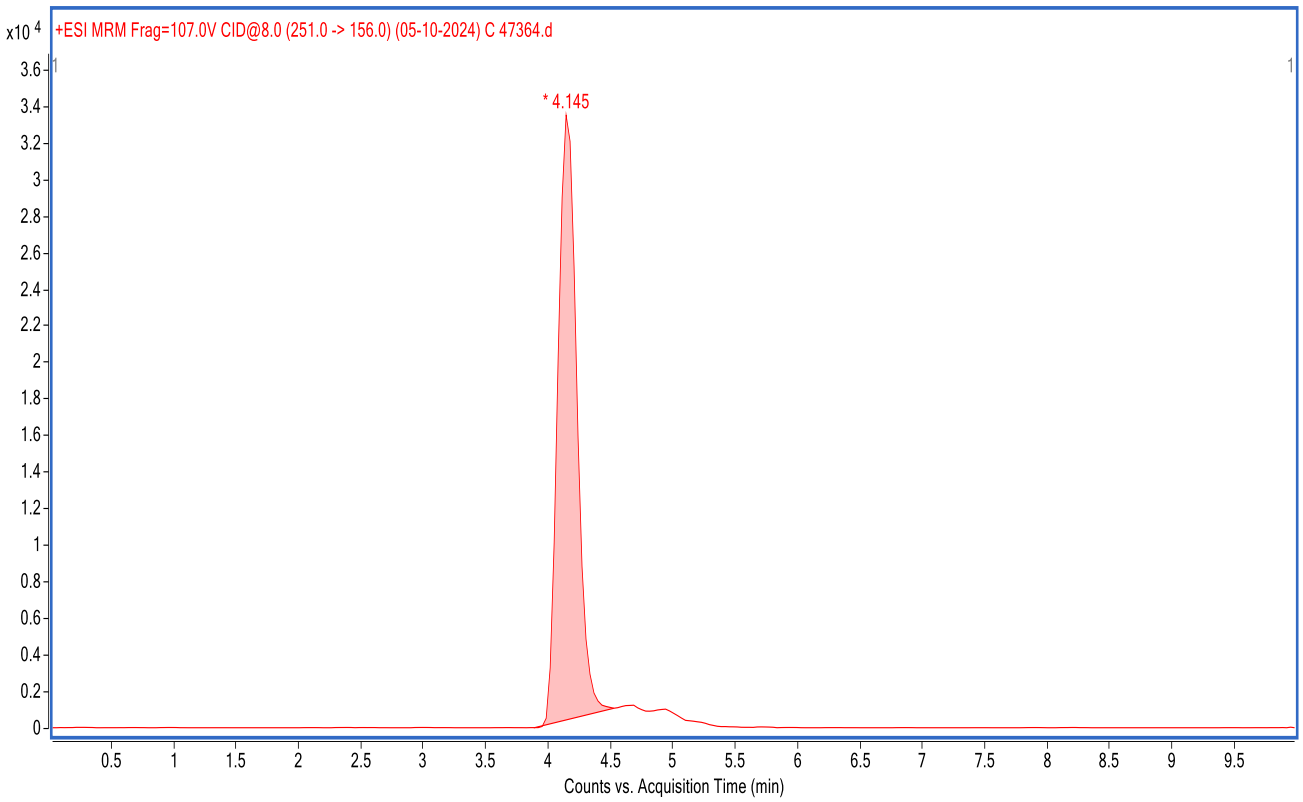


Fig 21: Sulfadiazin Quantifier ion

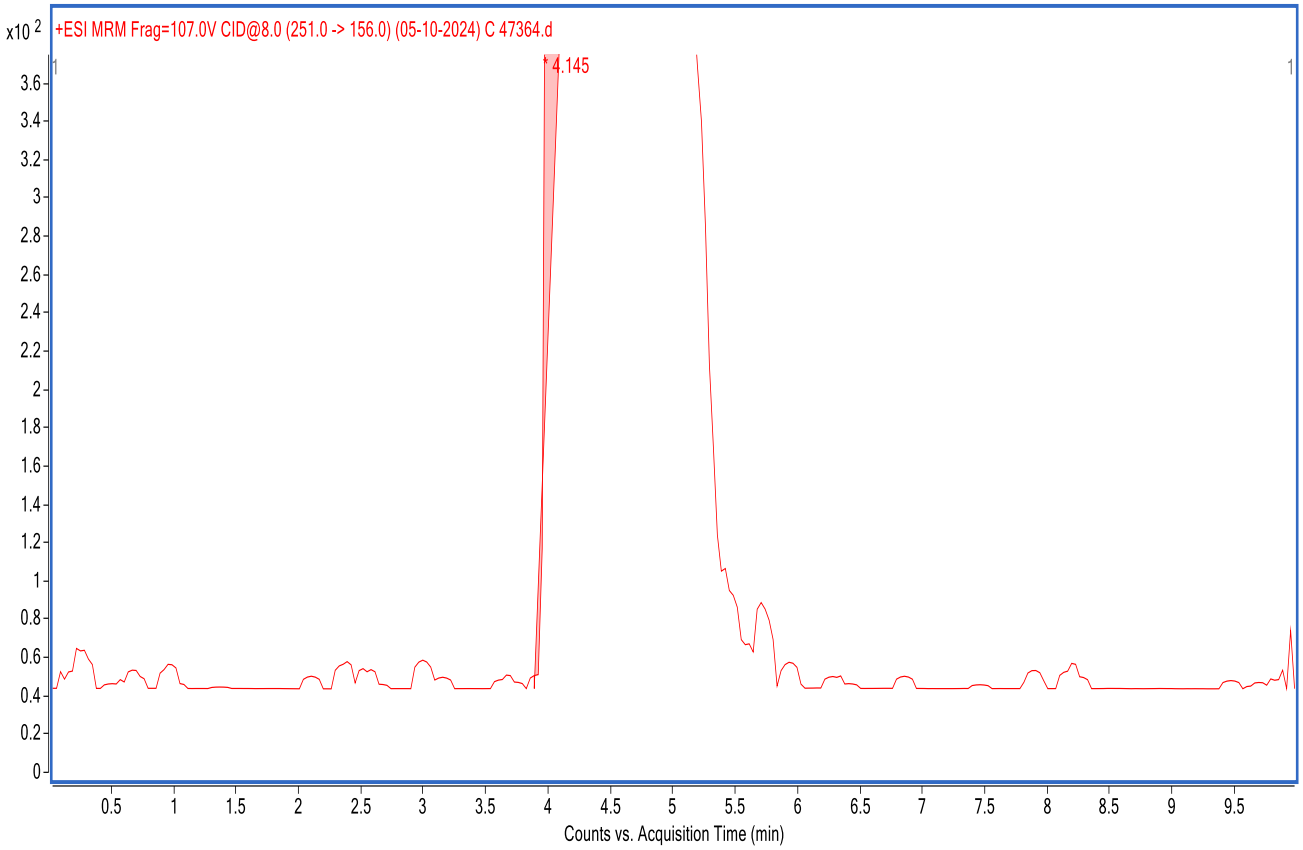


Fig 22: Sulfadiazin Quantifier ion zoomed view

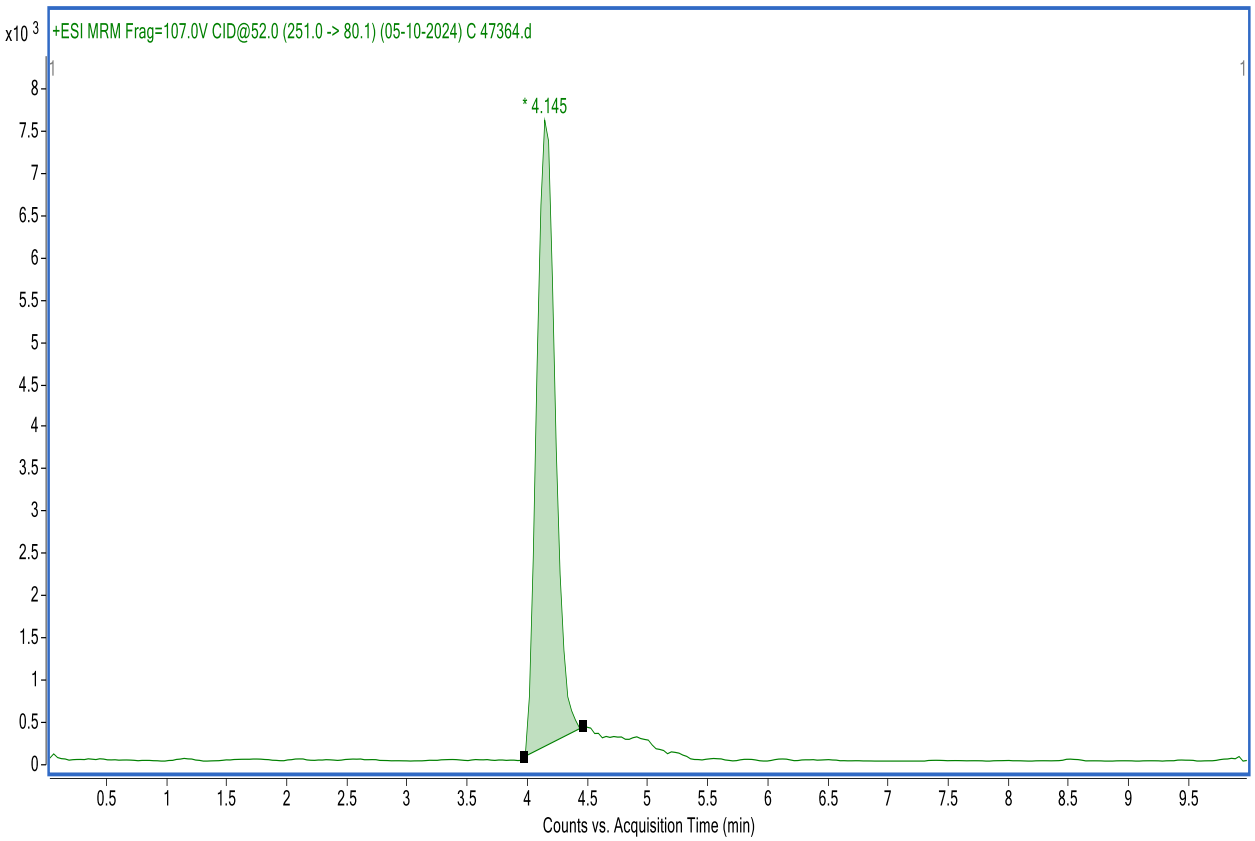


Fig 23: Sulfadiazin Qualifier ion

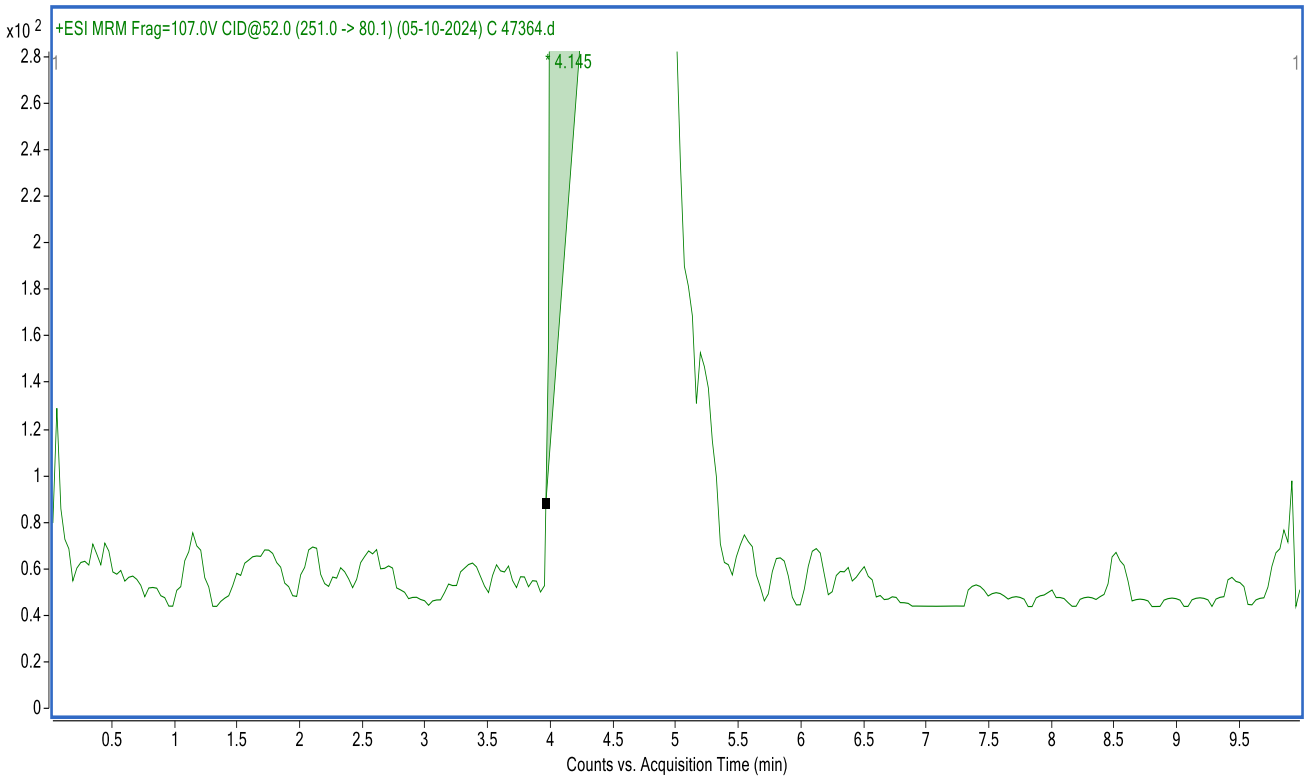


Fig 24: Sulfadiazin Qualifier ion zoomed view

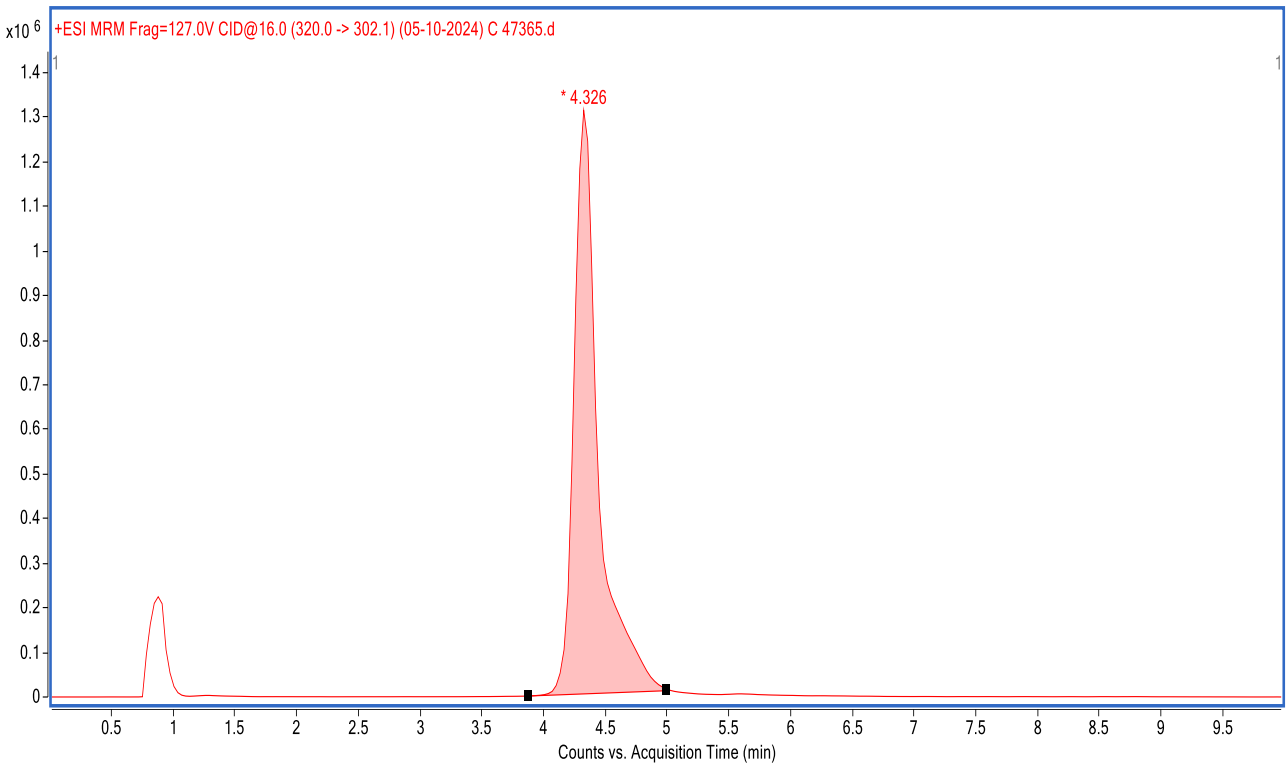


Fig 25: Norfloxacin Quantifier ion

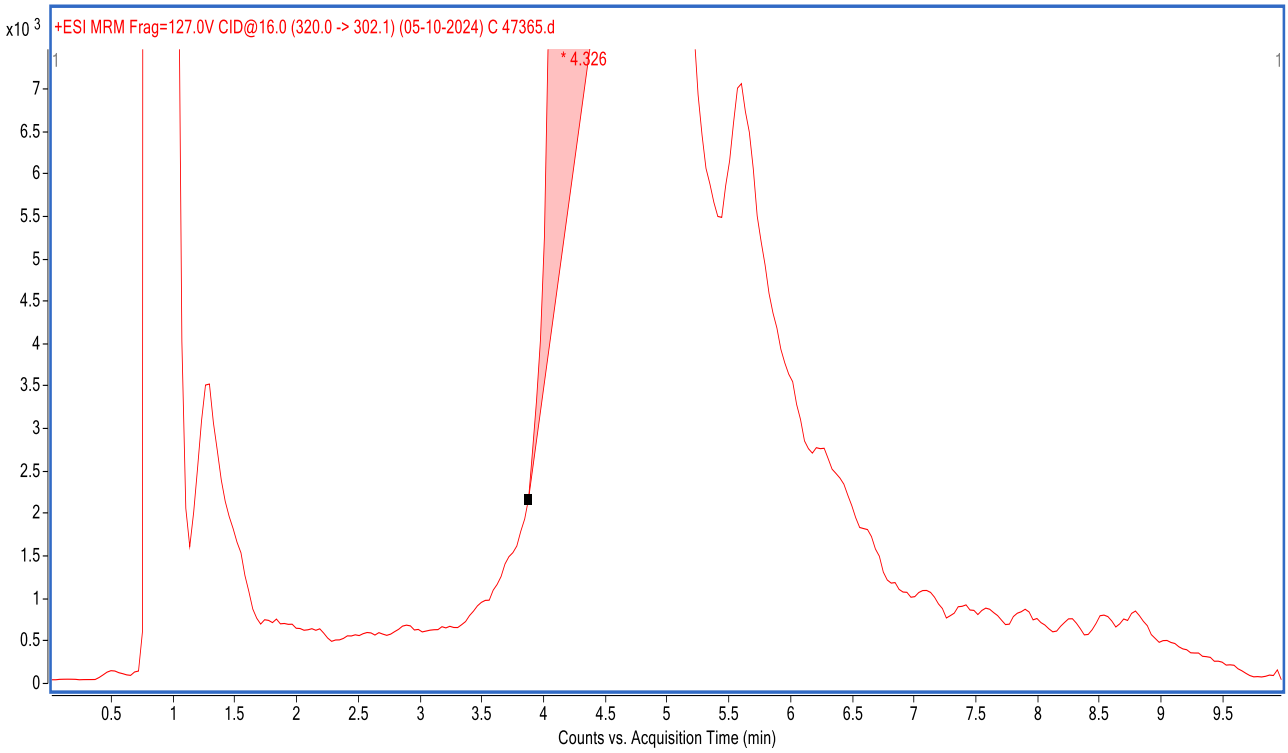


Fig 26: Norfloxacin Quantifier ion zoomed view

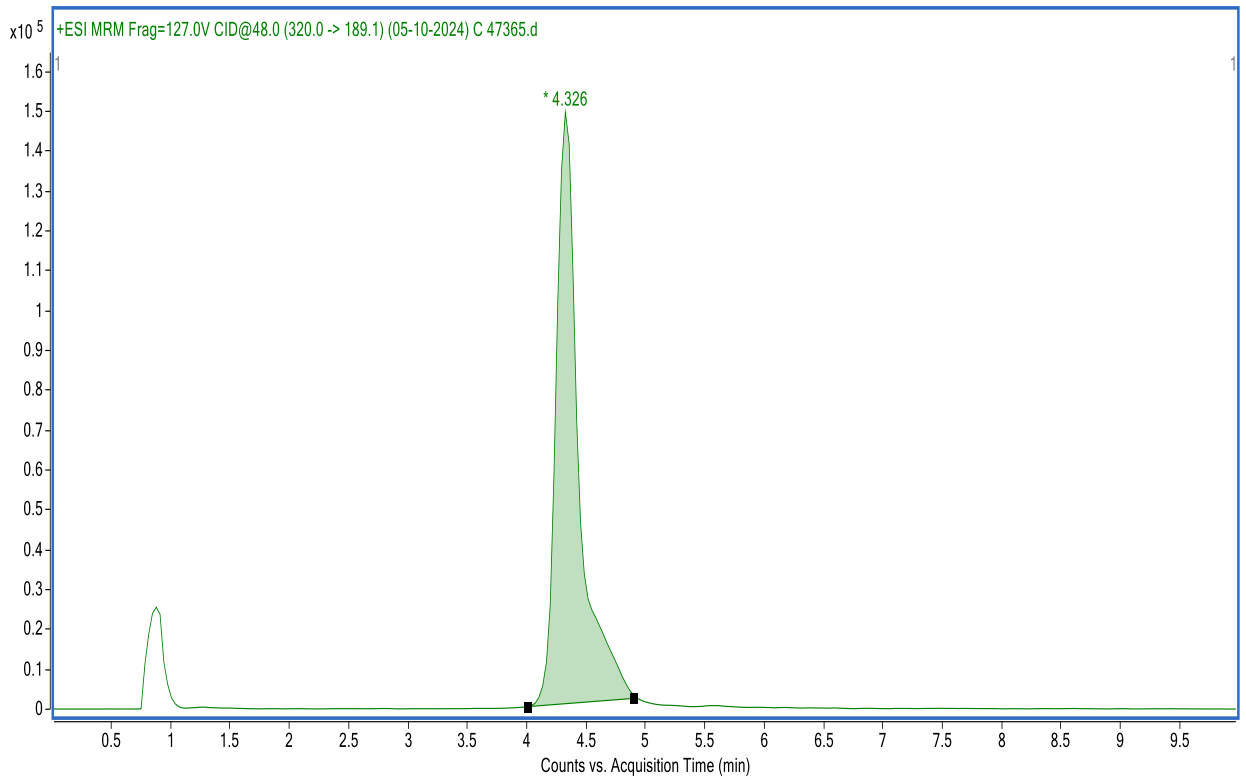


Fig 27: Norfloxacin Qualifier ion

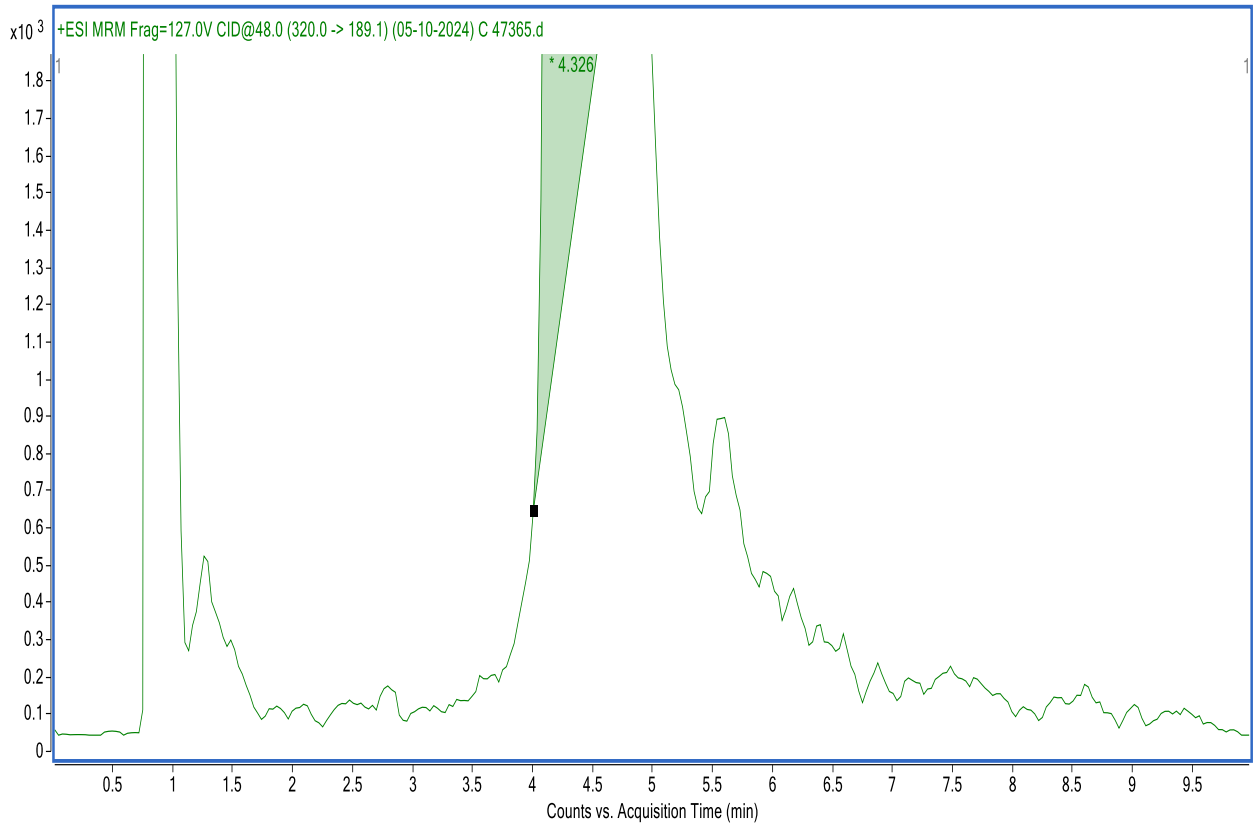


Fig 28: Norfloxacin Qualifier ion zoomed view

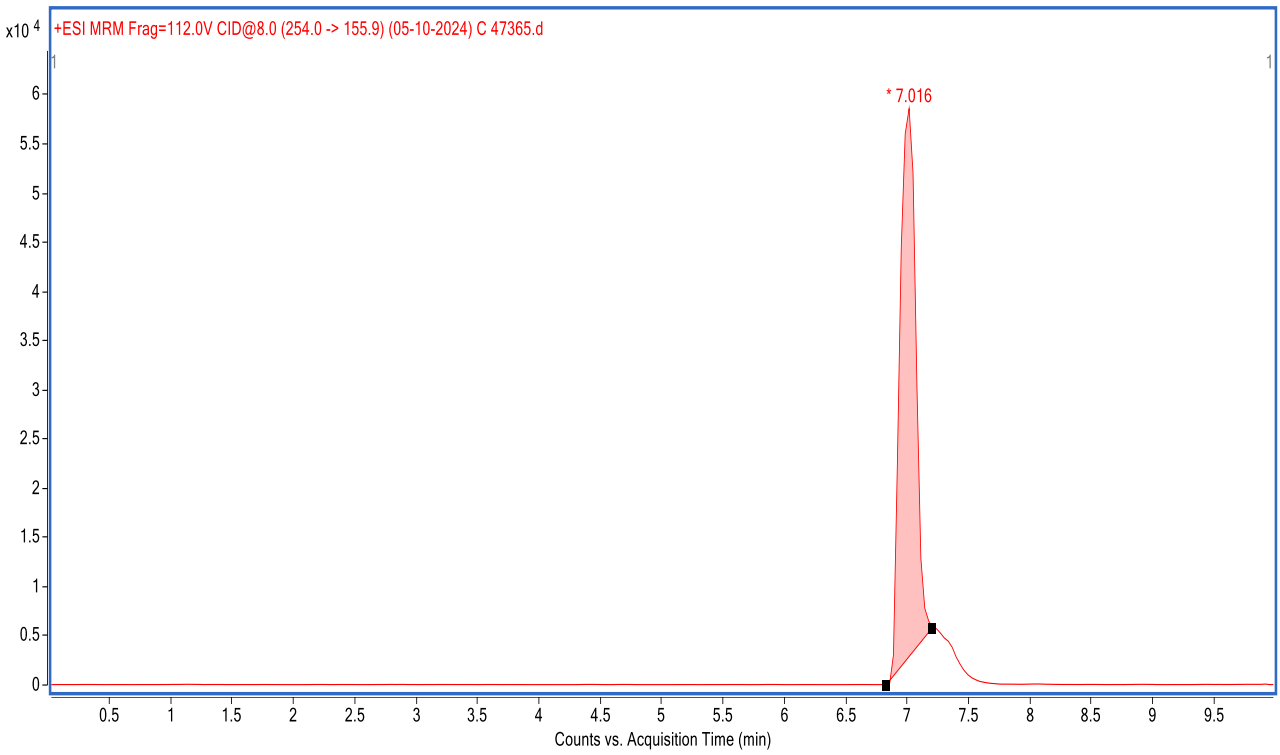


Fig 29: Sulfamethoxazole Quantifier ion

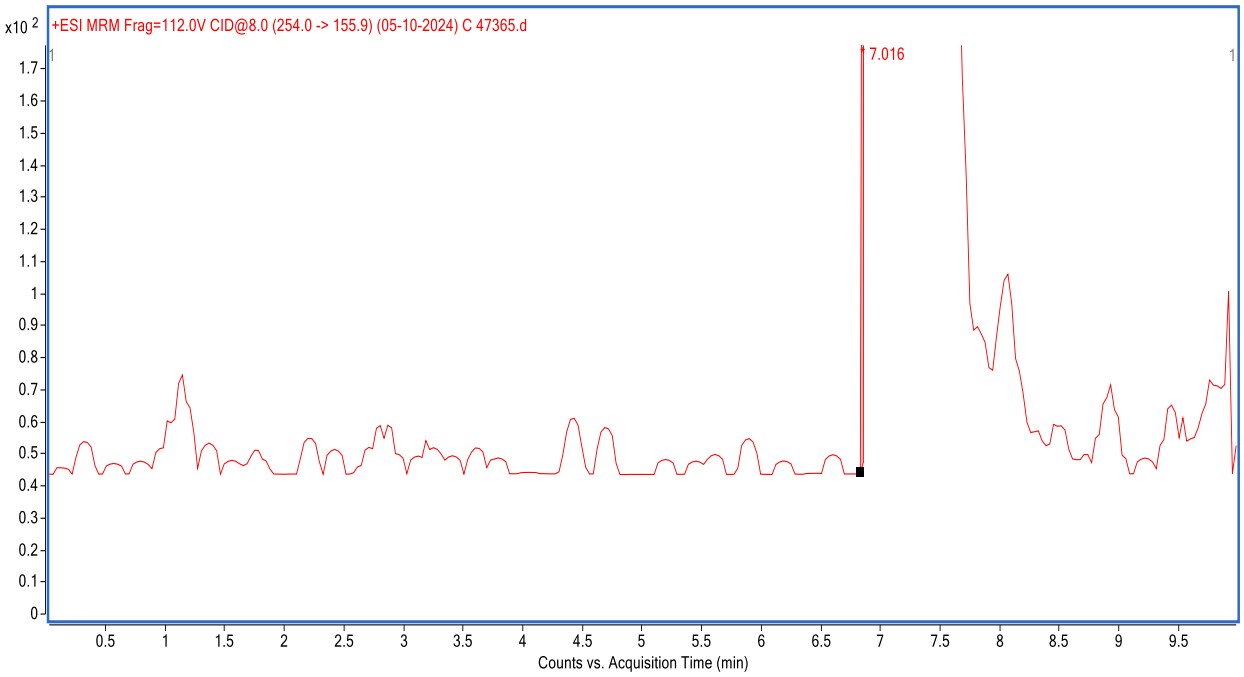


Fig 30: Sulfamethoxazole Quantifier ion Zoomed ion

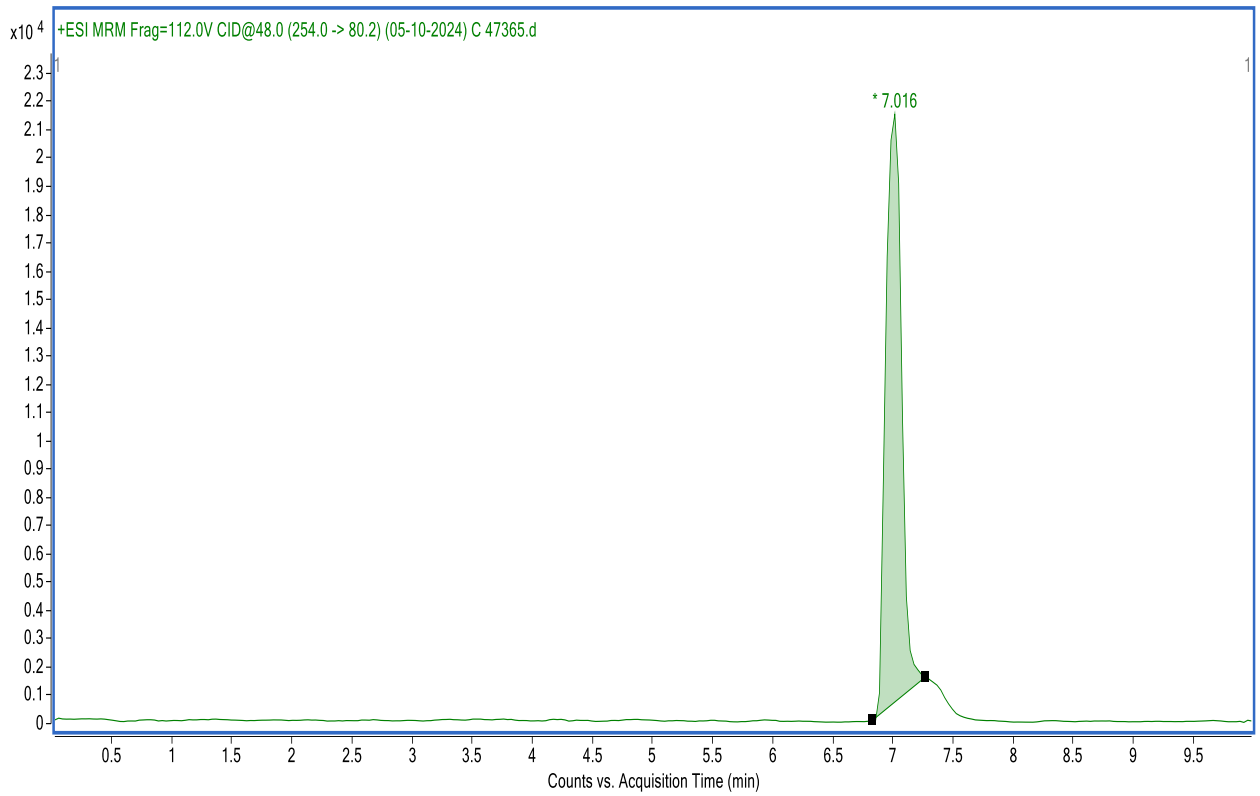


Fig 31: Sulfamethoxazole Qualifier ion

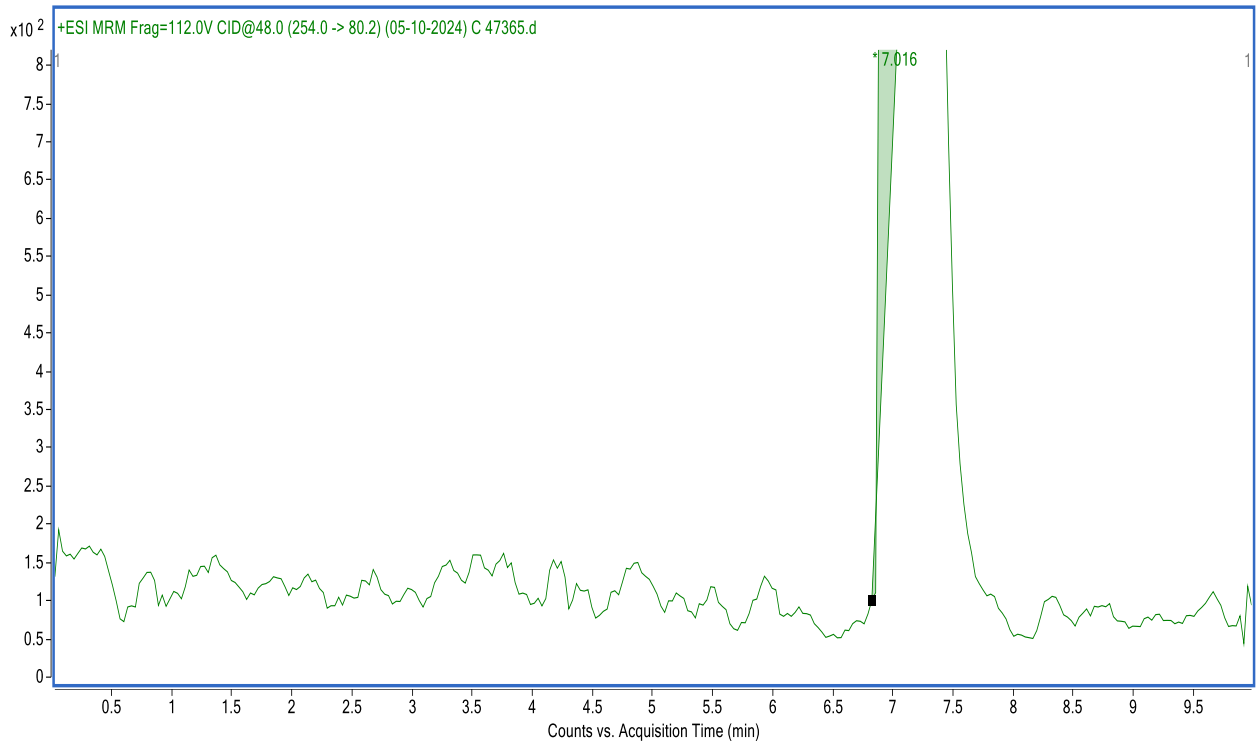


Fig 32: Sulfamethoxazole Qualifier ion Zoomed view

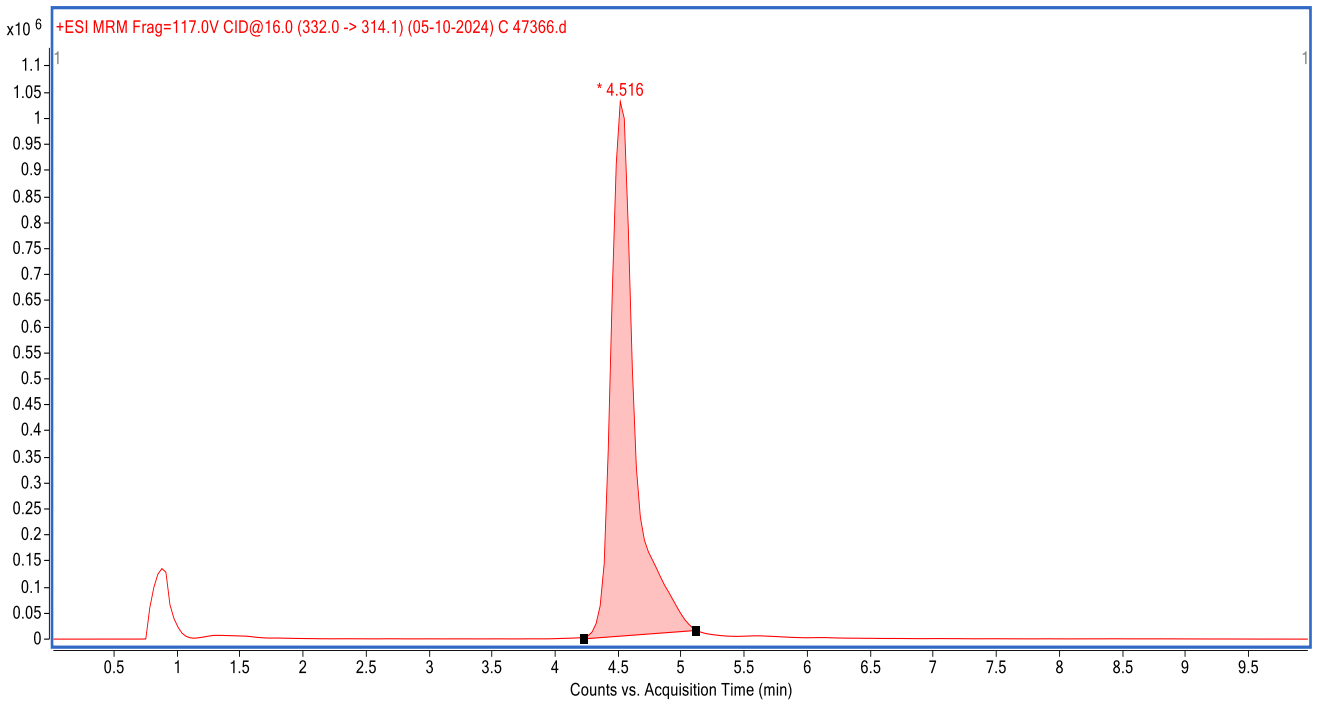


Fig 33: Ciprofloxacin Quantifier ion

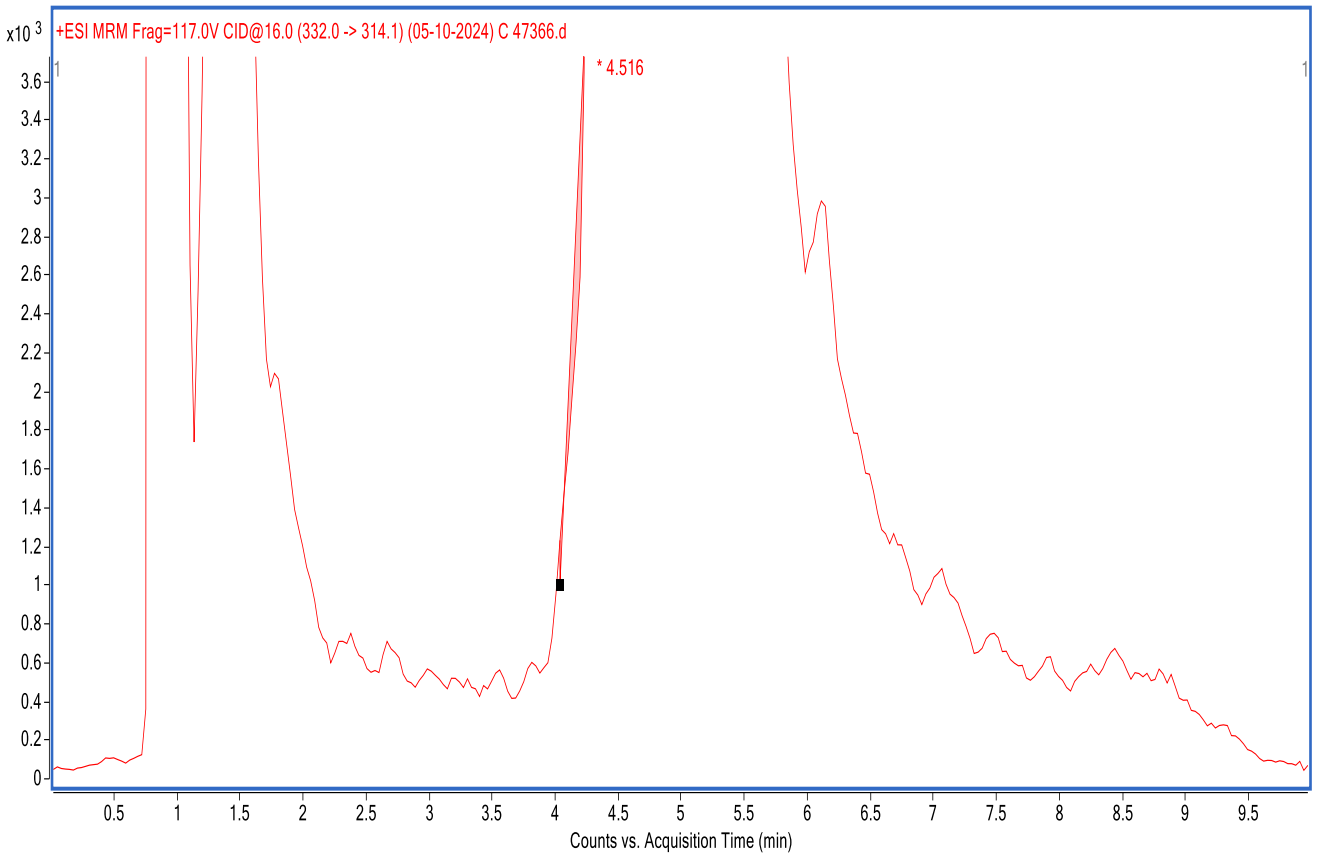


Fig 34: Ciprofloxacin Quantifier ion Zoomed view

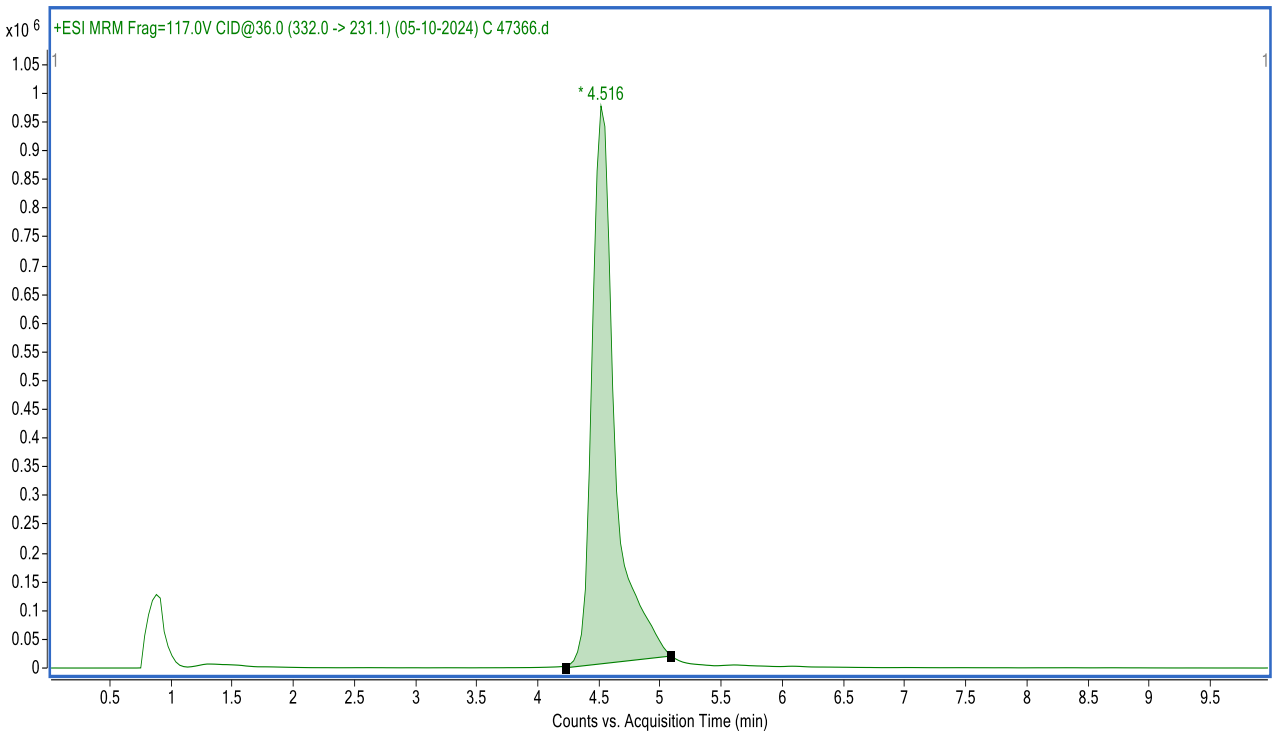


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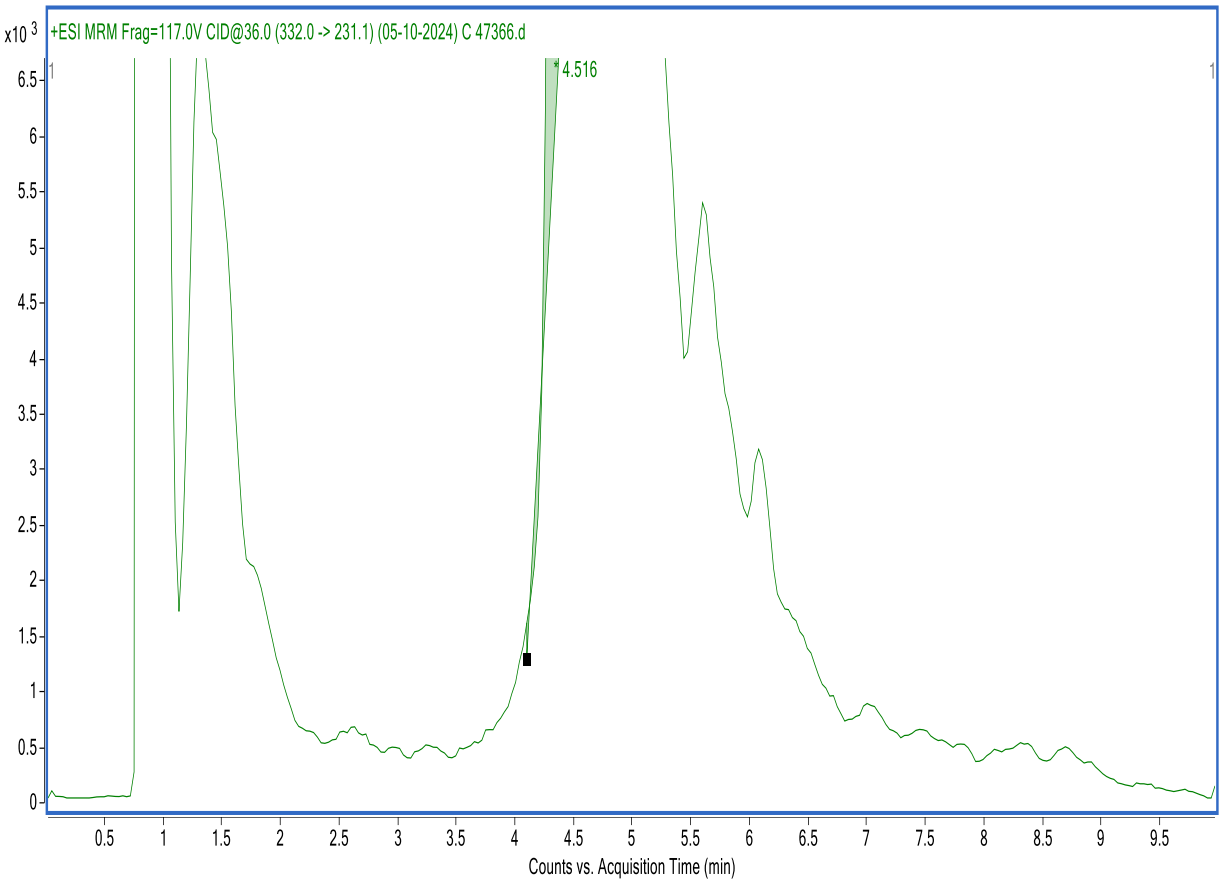


Fig 36: Ciprofloxacin Qualifier ion Zoomed view

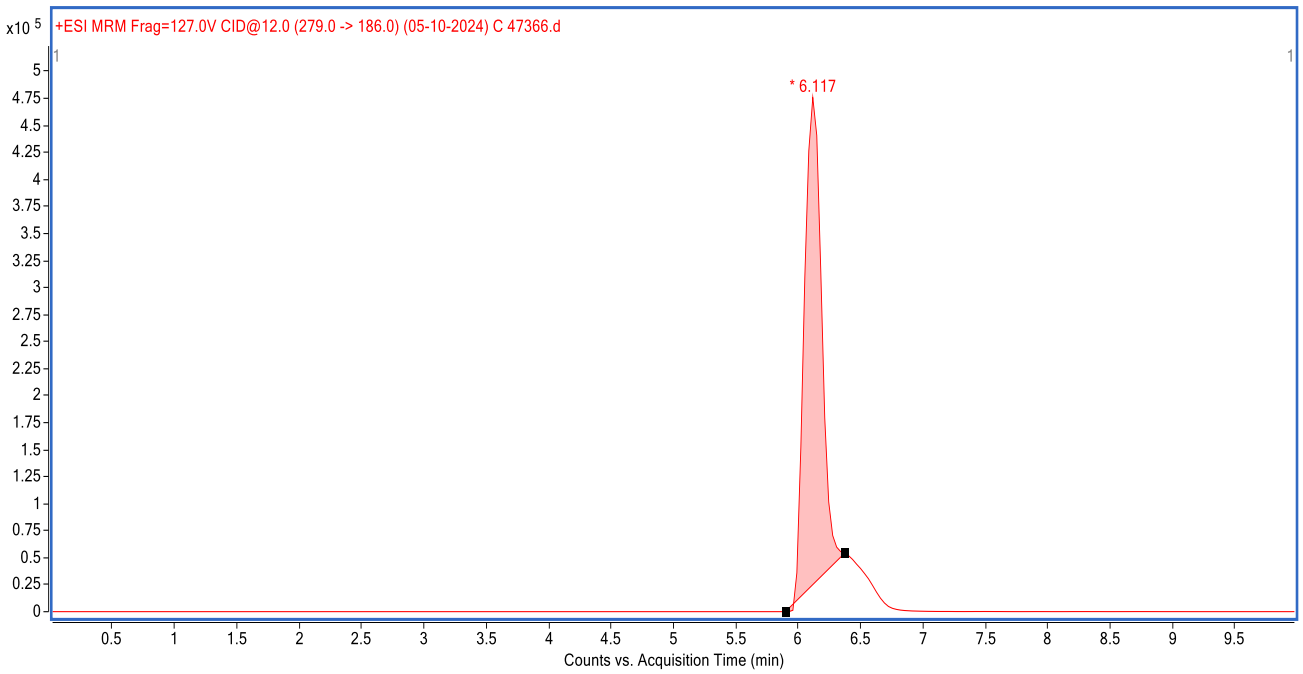


Fig 37: Sulfadimidin Quantifier ion

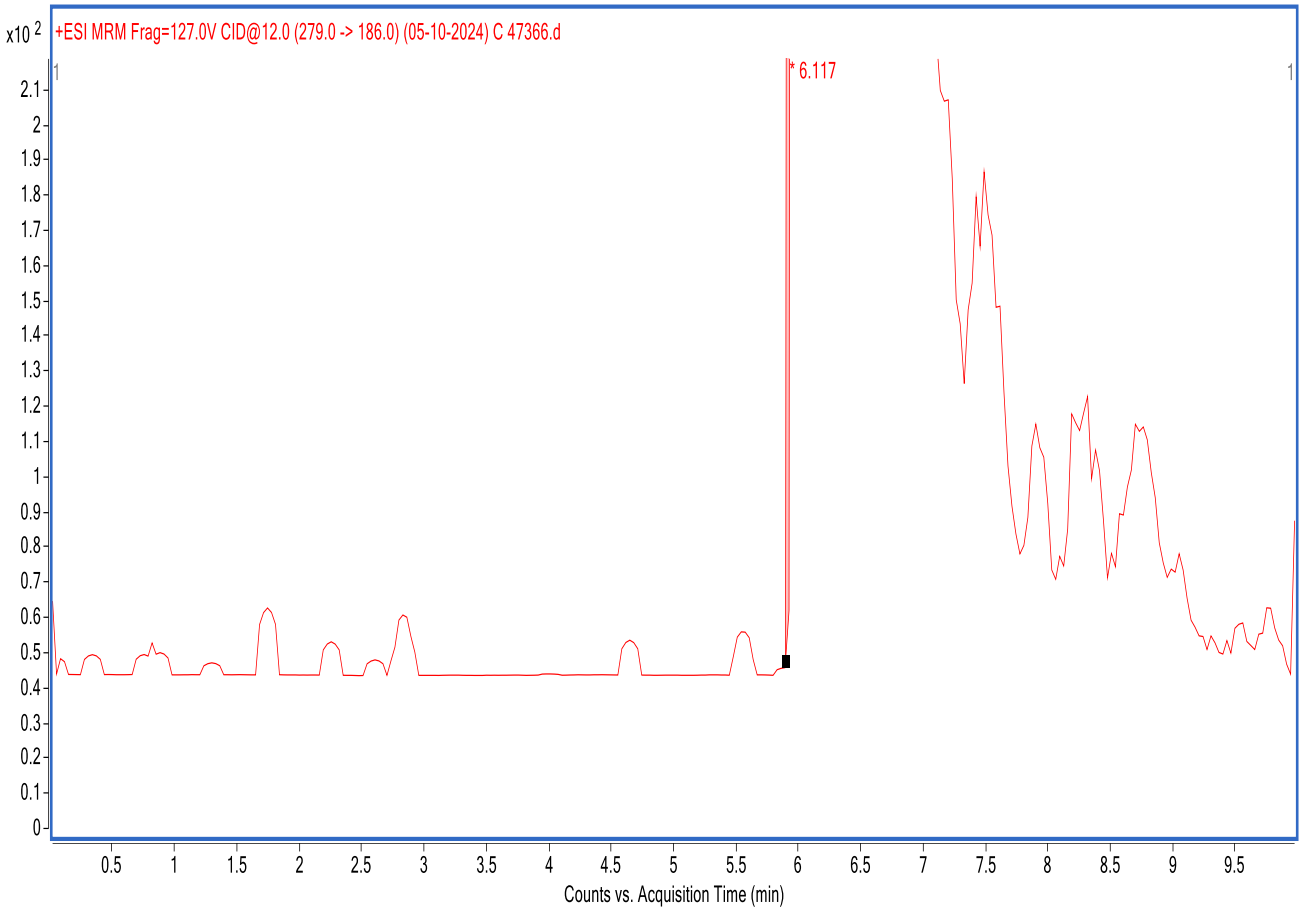


Fig 38: Sulfadimidin Quantifier ion Zoomed view

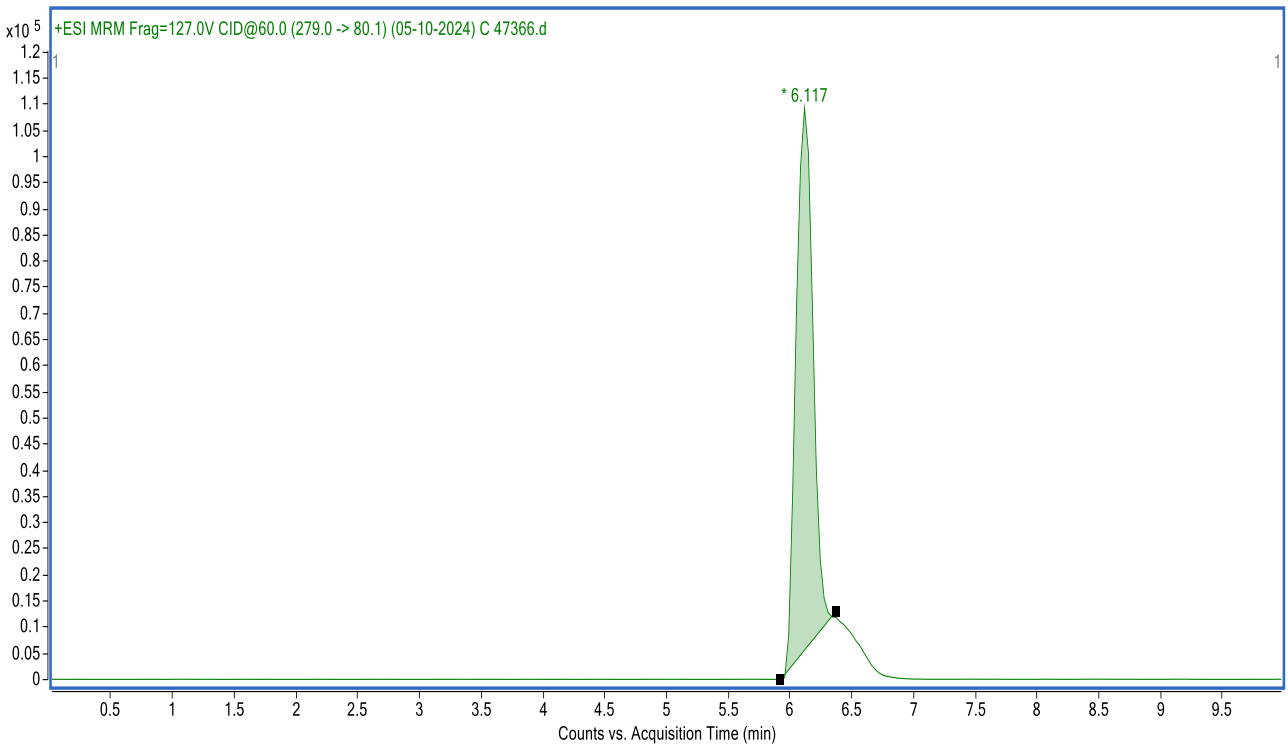


Fig 39: Sulfadimidin Qualifier ion

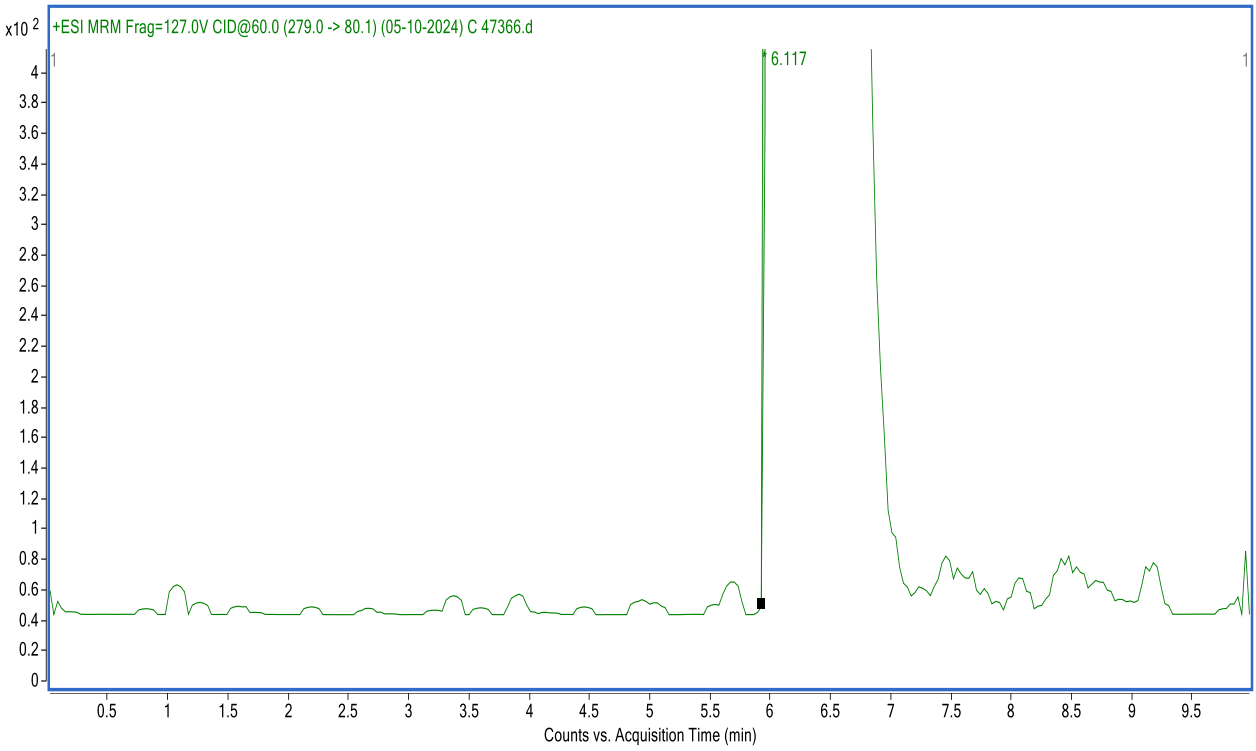


Fig 40: Sulfadimidin Qualifier ion Zoomed view

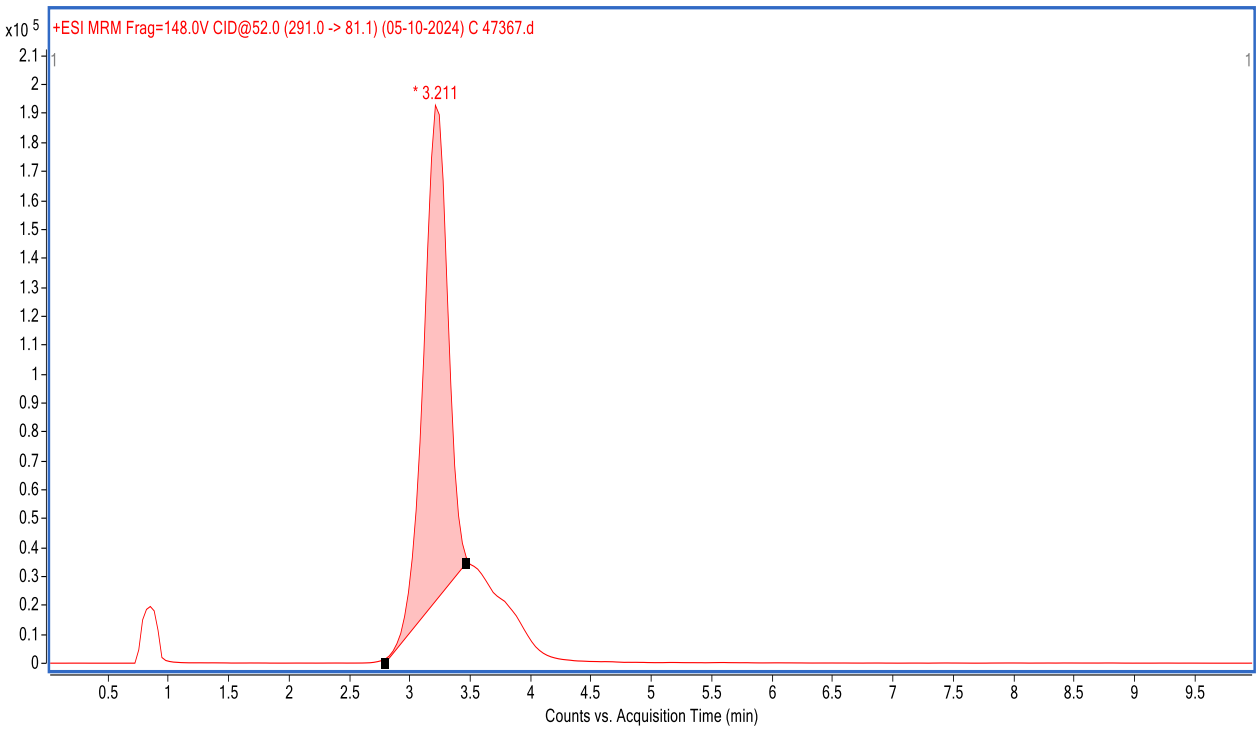


Fig 41: Trimethoprim Quantifier ion

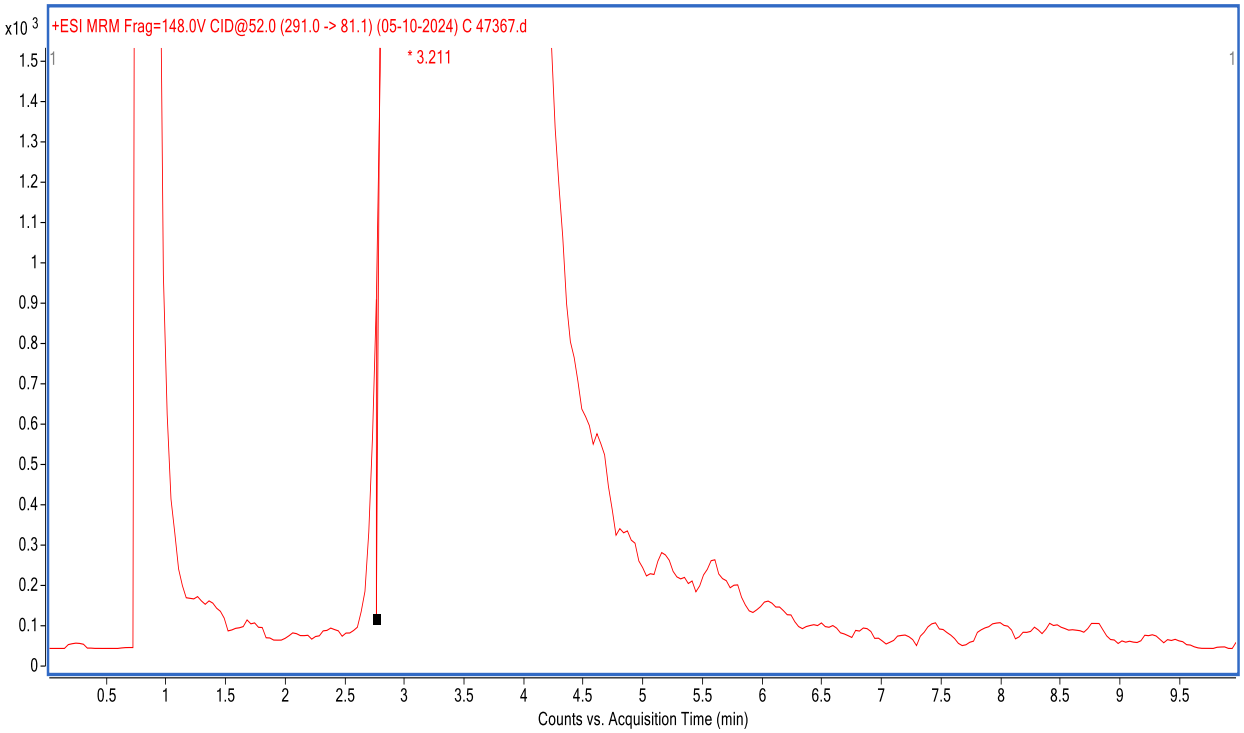


Fig 42: Trimethoprim Quantifier ion Zoomed view

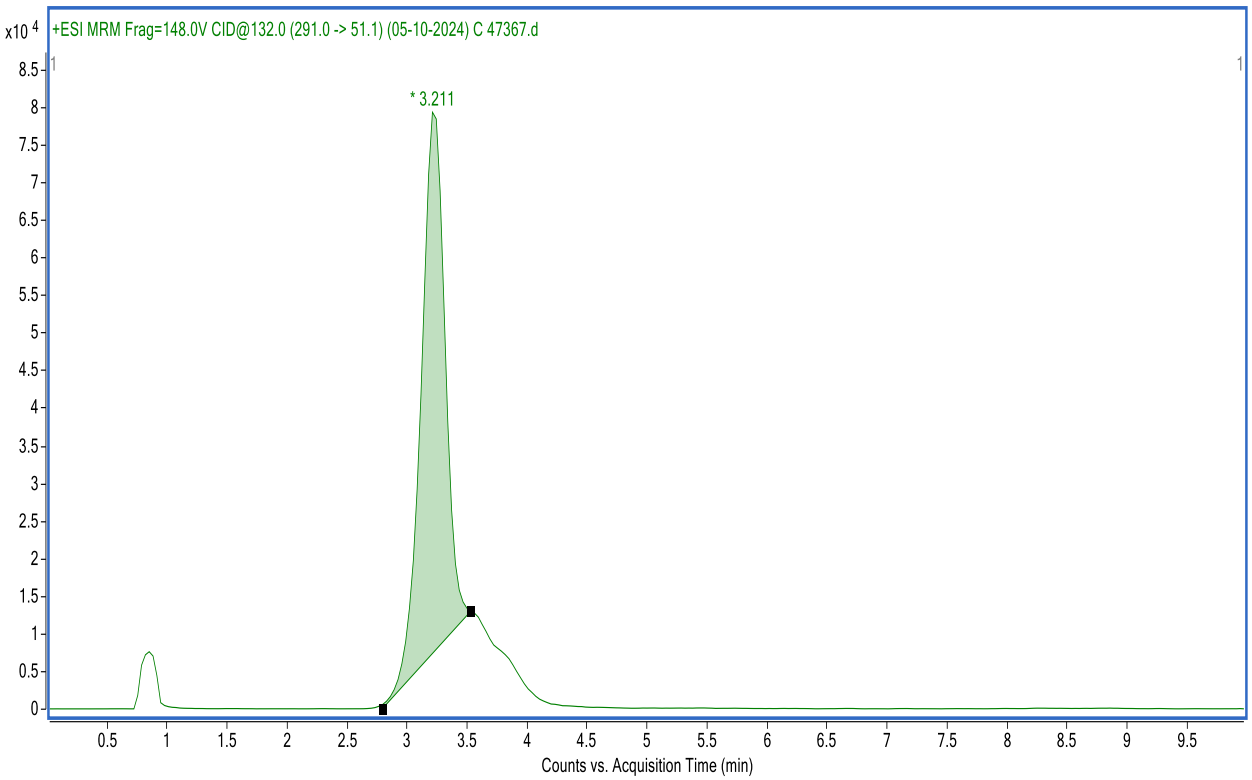


Fig 43: Trimethoprim Qualifier ion

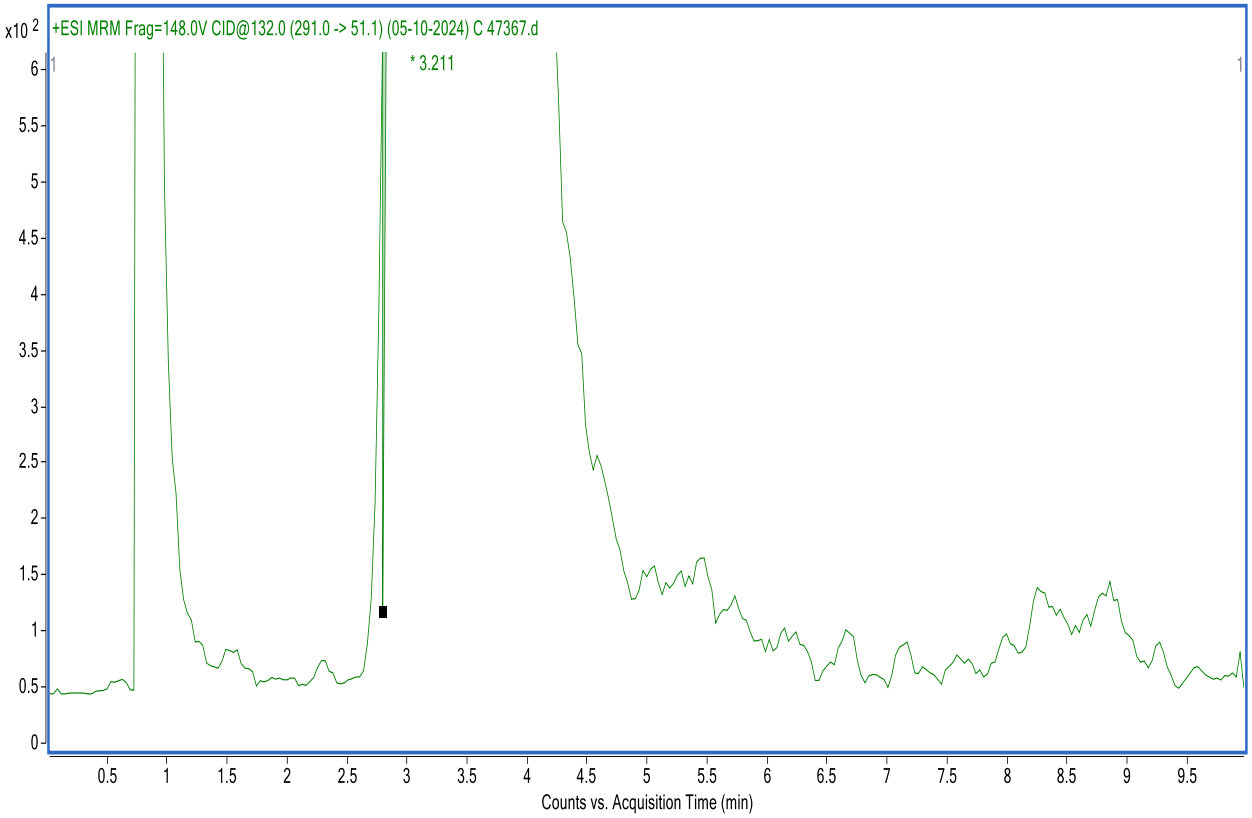


Fig 44: Trimethoprim Qualifier ion Zoomed view

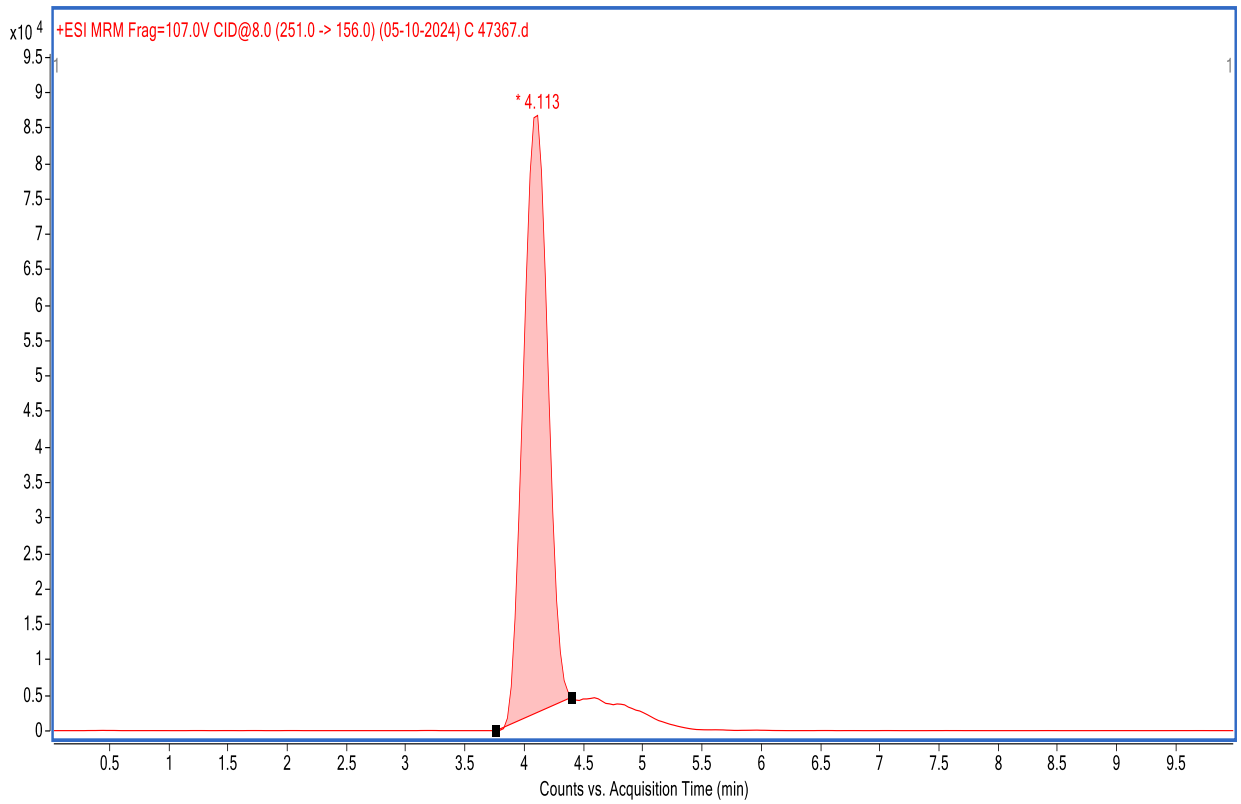


Fig 45: Sulfadiazin Quantifier ion

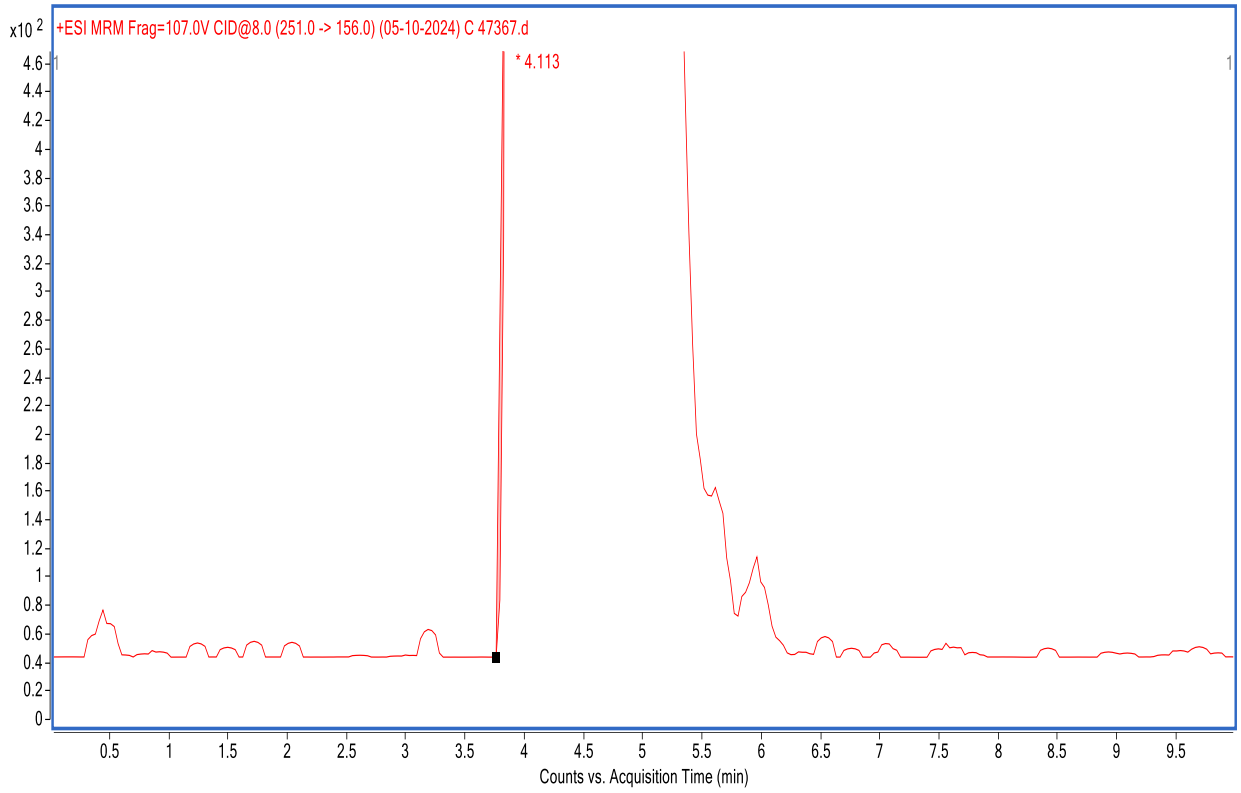


Fig 46: Sulfadiazin Quantifier ion Zoomed view

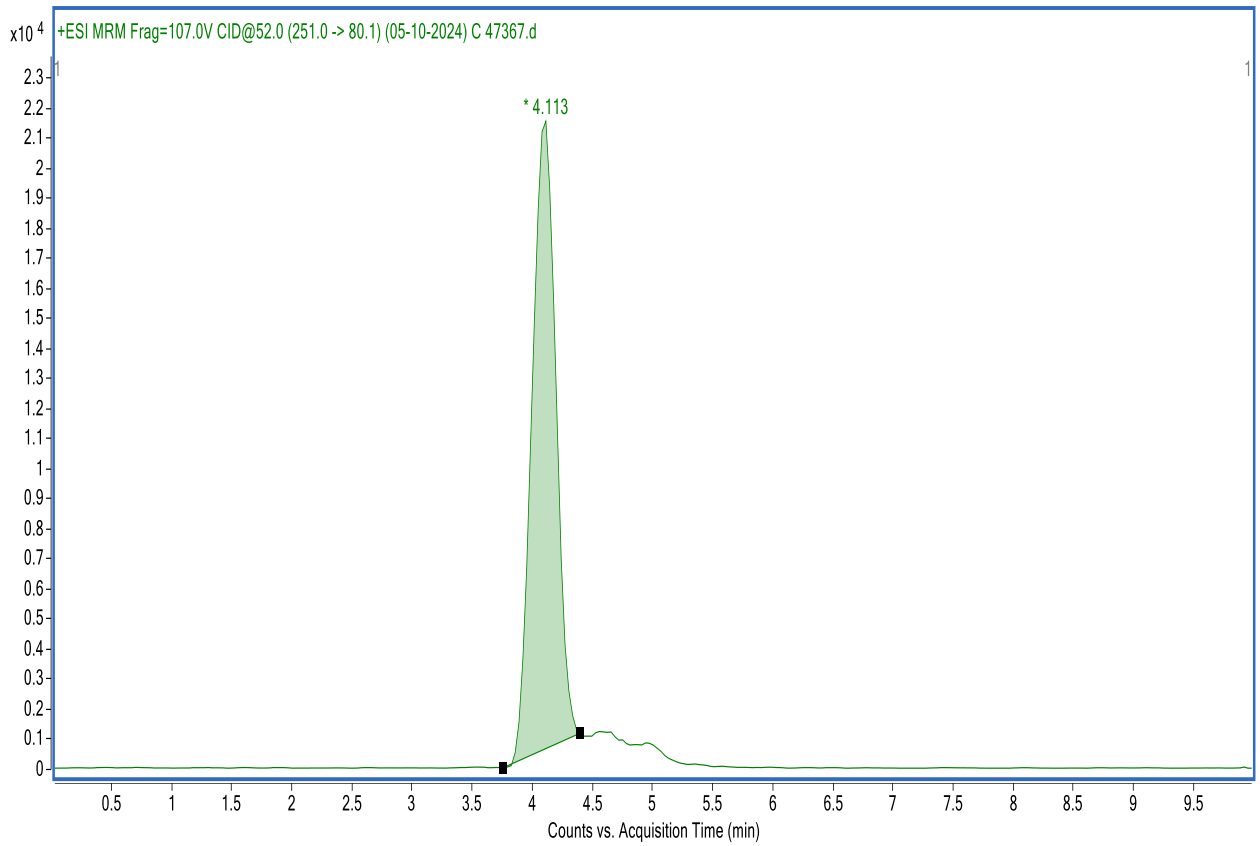


Fig 47: Sulfadiazin Qualifier ion

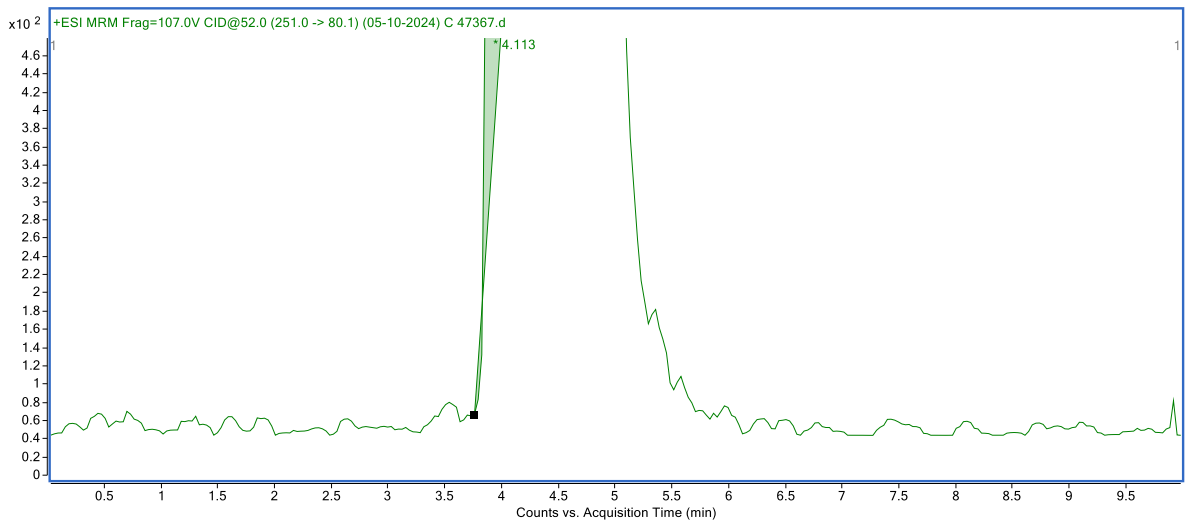


Fig 48: Sulfadiazin Qualifier ion Zoomed view

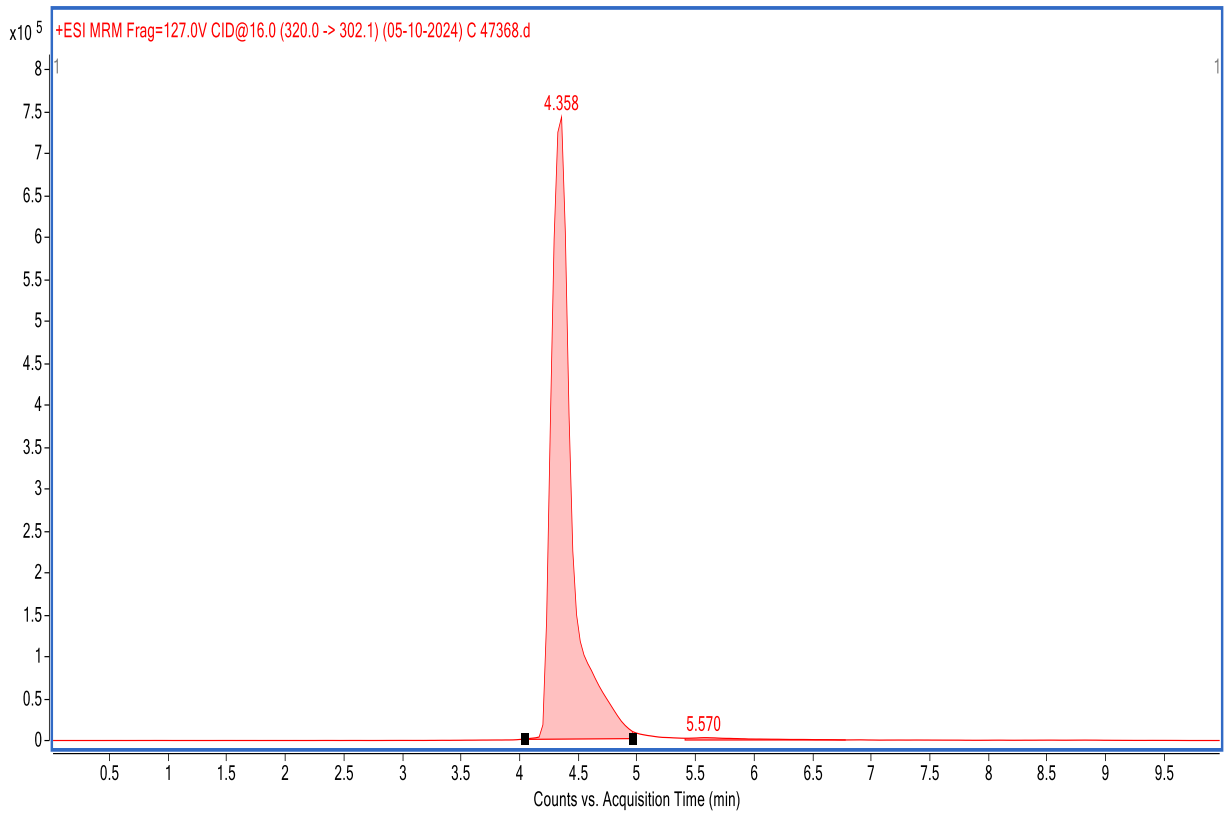


Fig 49: Norfloxacin Quantifier ion

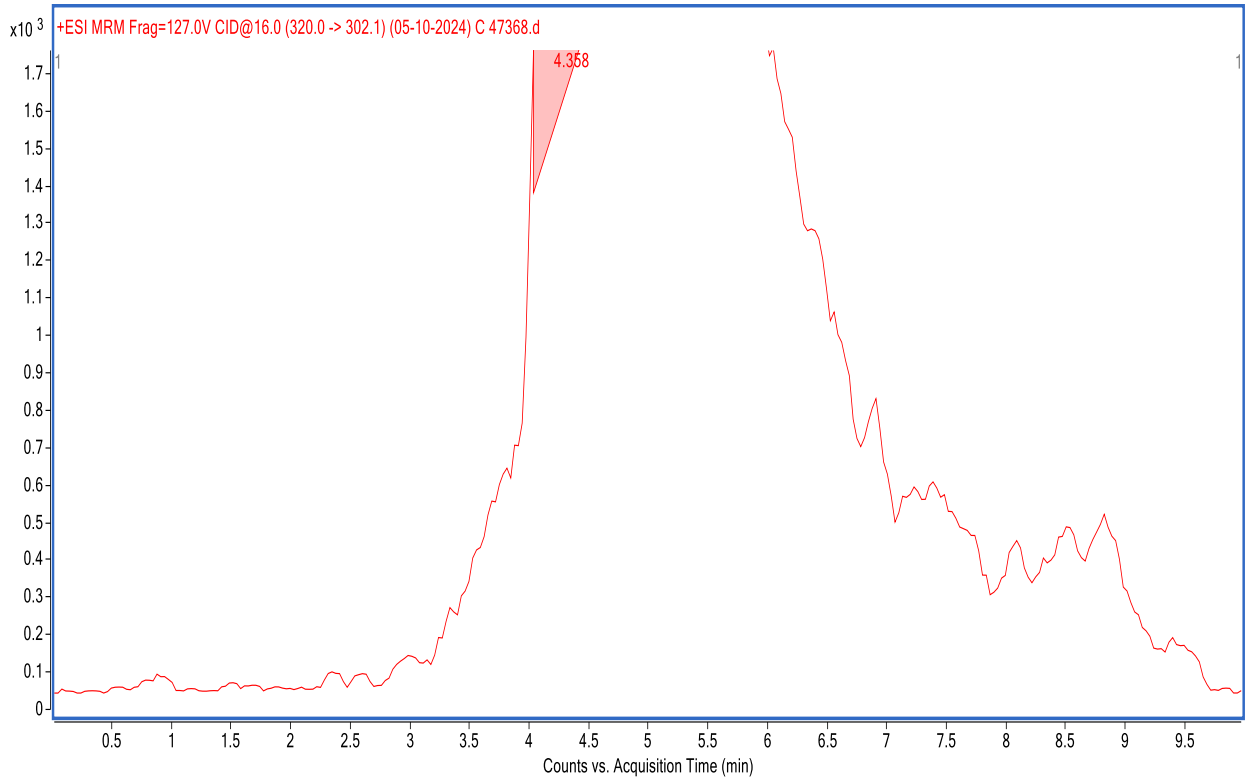


Fig 50: Norfloxacin Quantifier ion Zoomed view

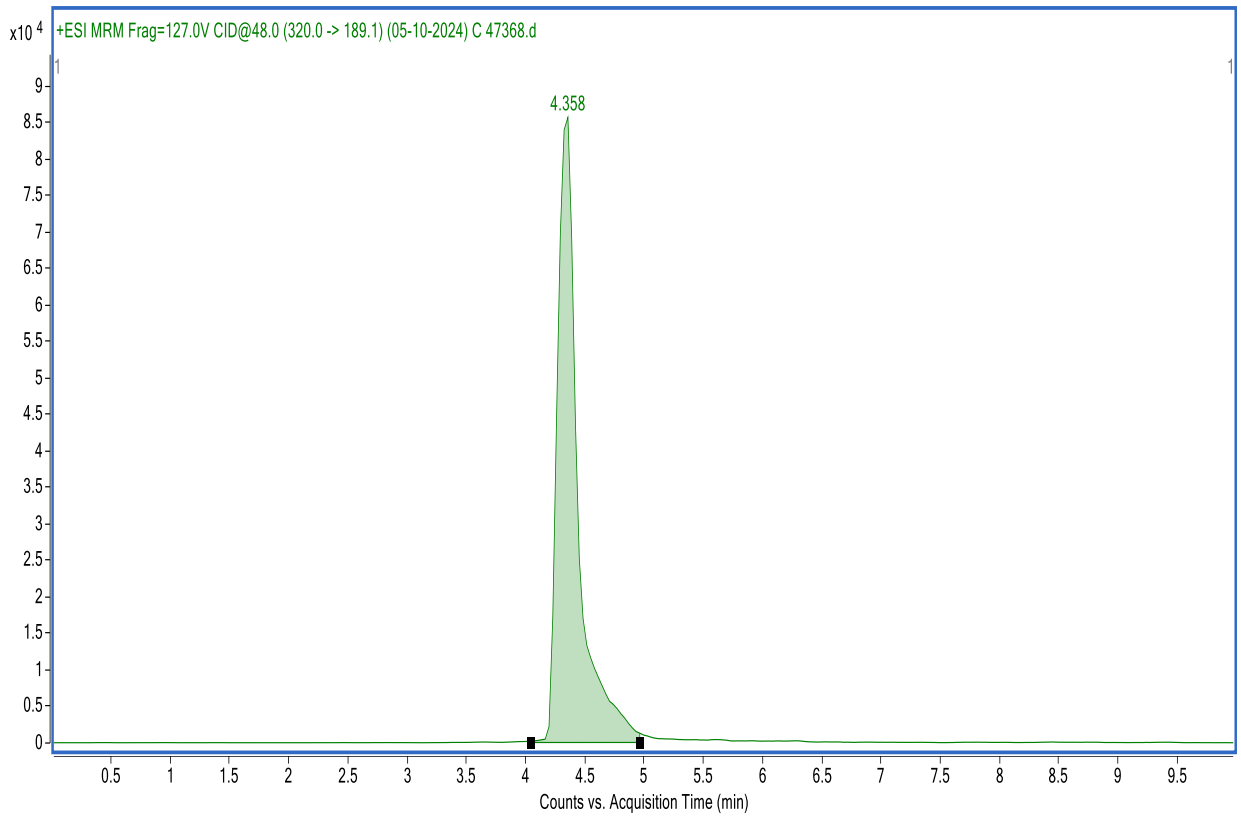


Fig 51: Norfloxacin Qualifier ion

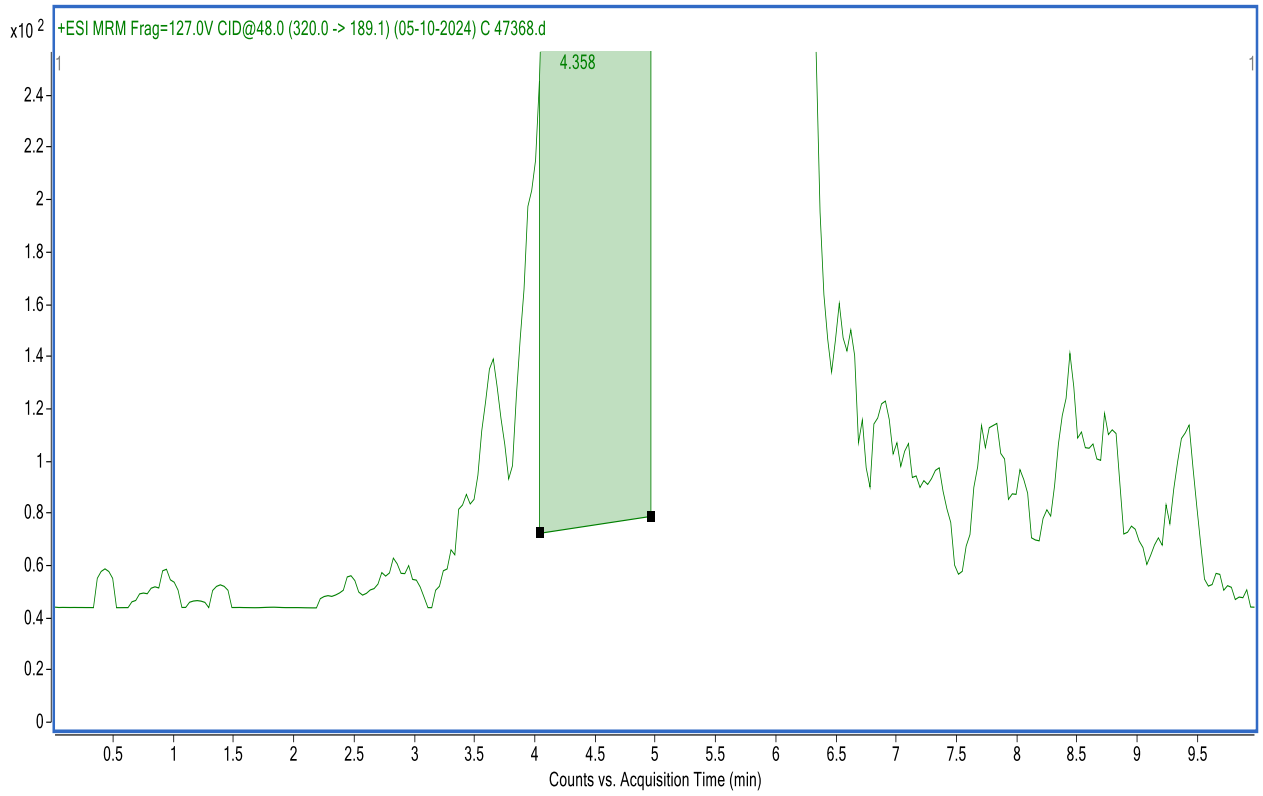


Fig 52: Norfloxacin Qualifier ion Zoomed view

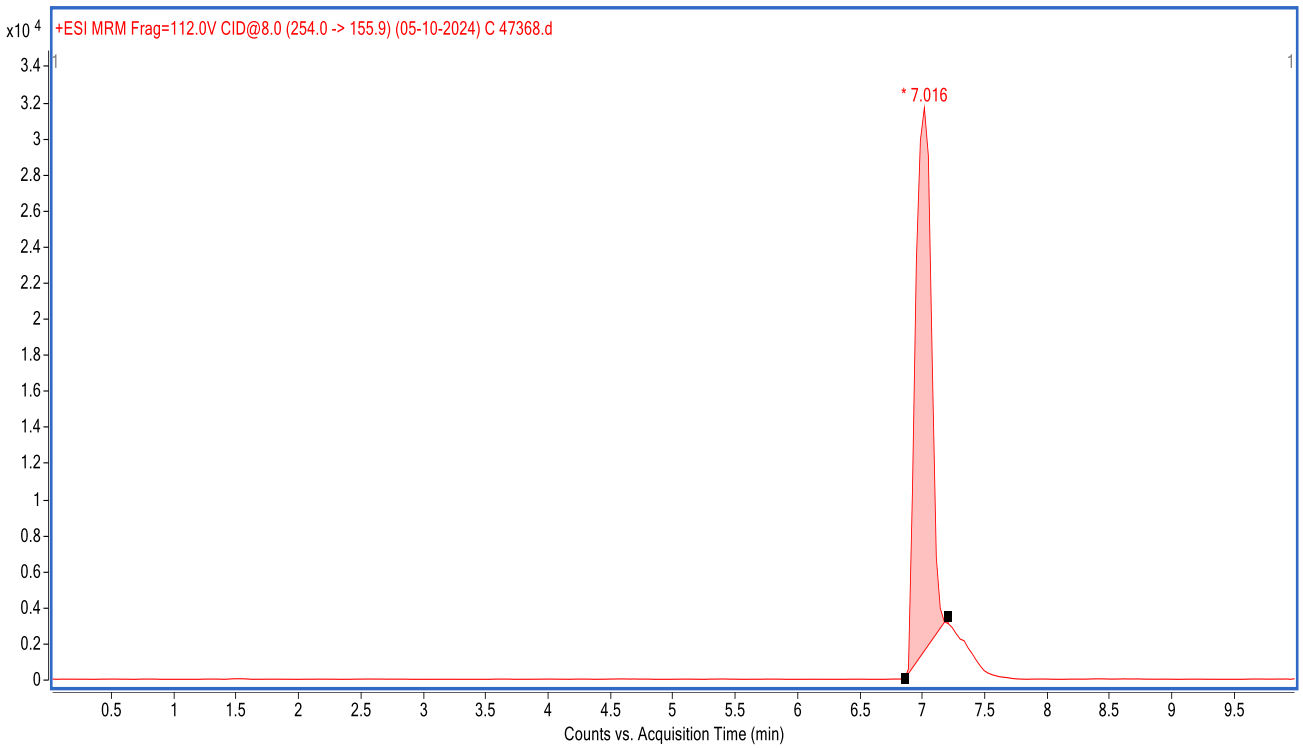


Fig 53: Sulfamethoxazole Quantifier ion

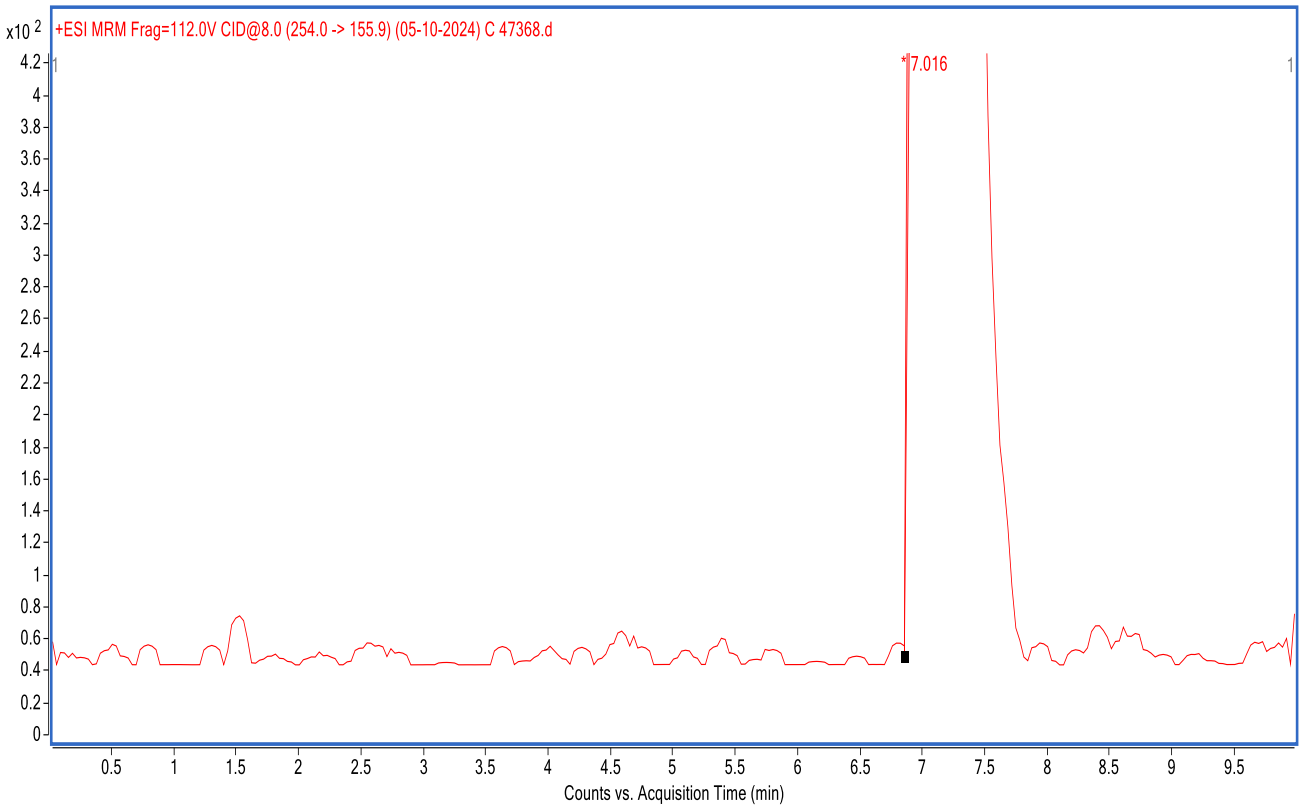


Fig 54: Sulfamethoxazole Quantifier ion Zoomed view

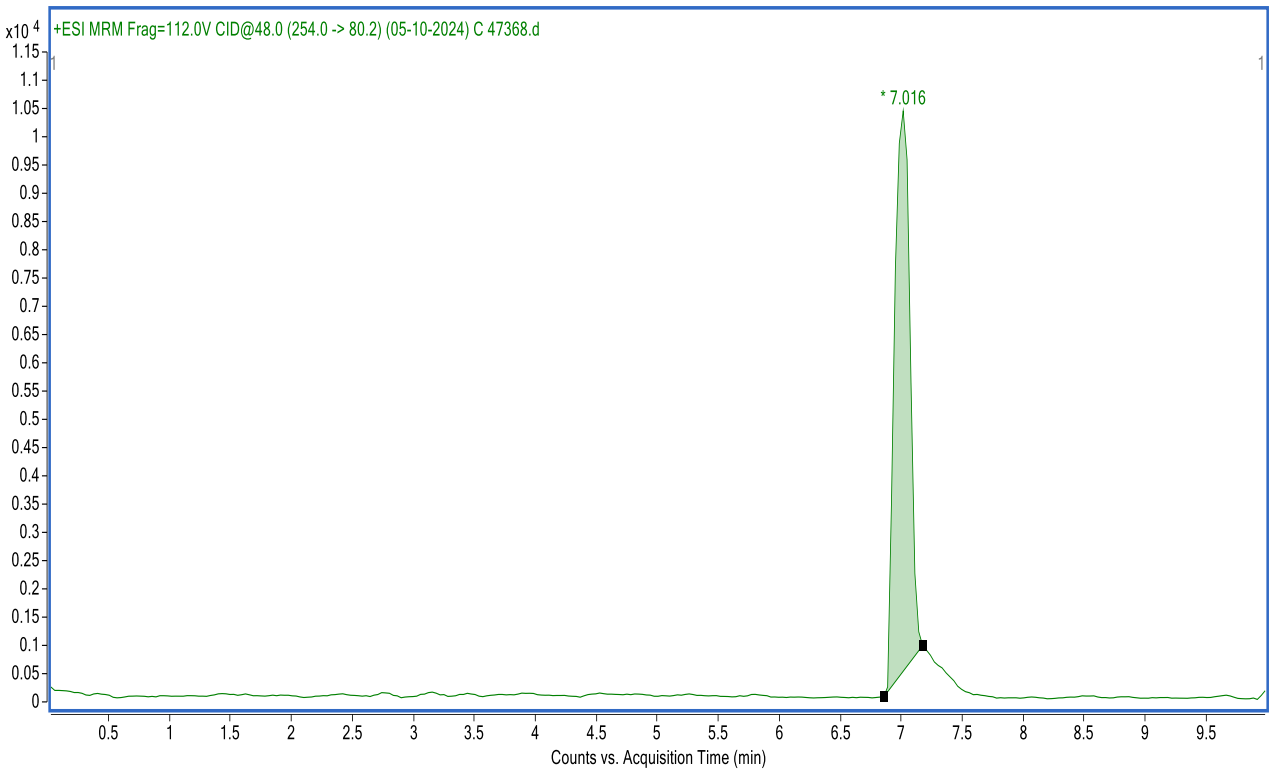


Fig 55: Sulfamethoxazole Qualifier ion

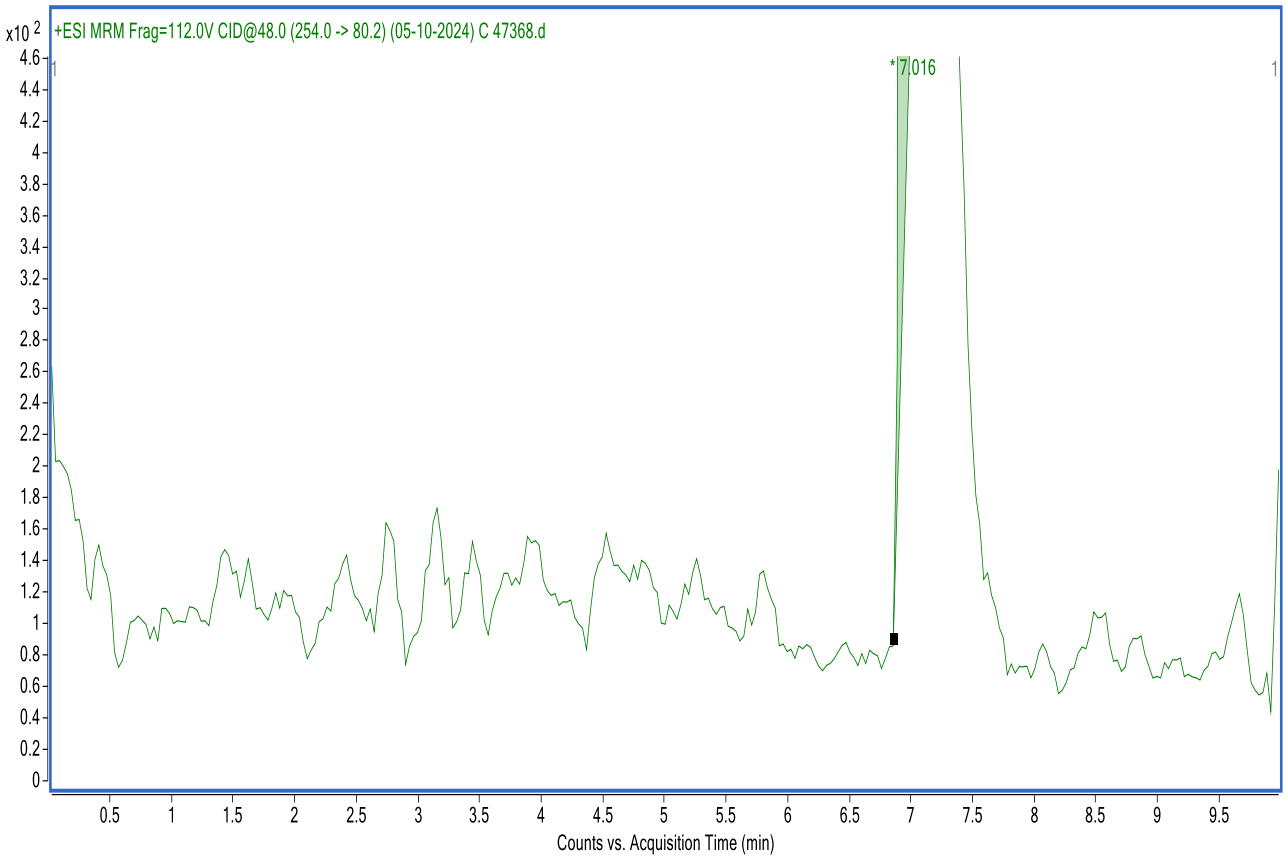


Fig 56: Sulfamethoxazole Qualifier ion Zoomed view

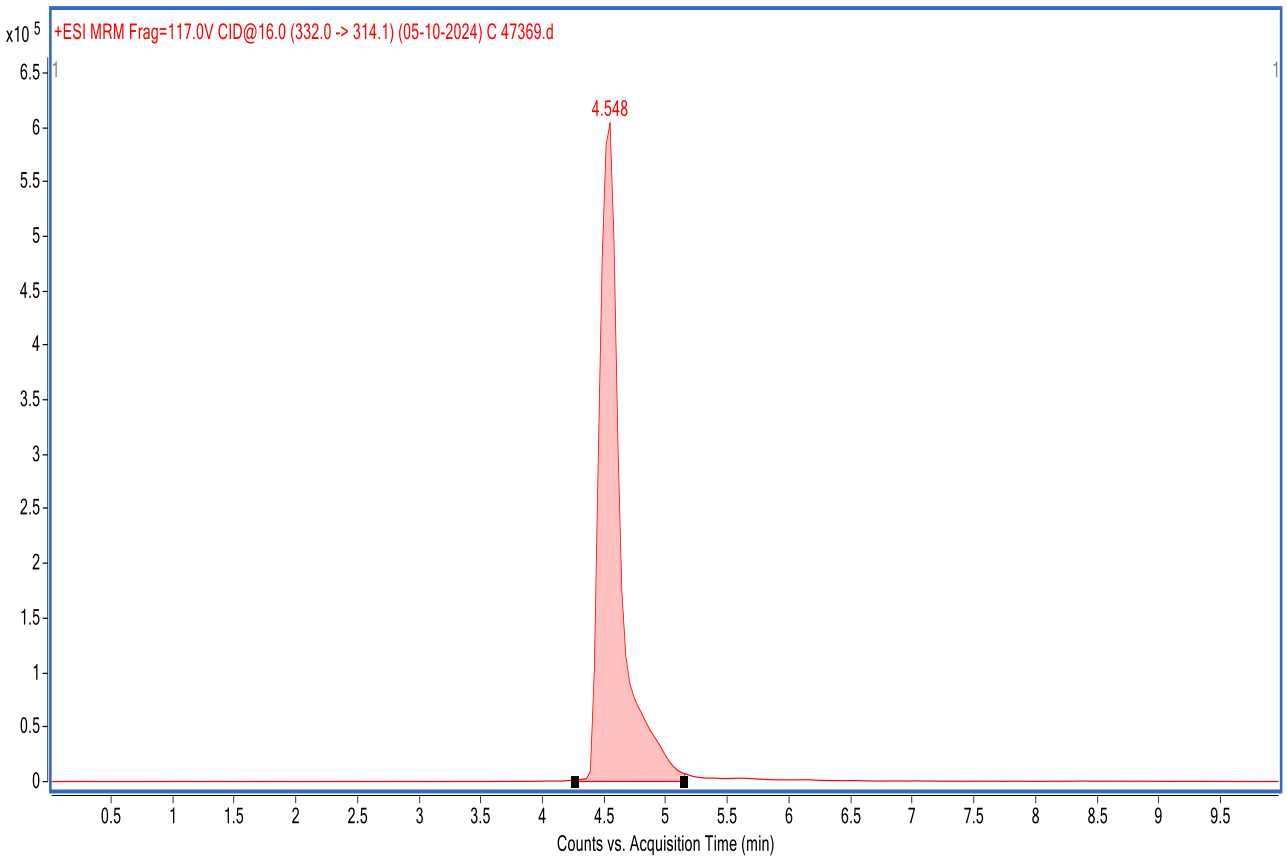


Fig 57: Ciprofloxacin Quantifier ion

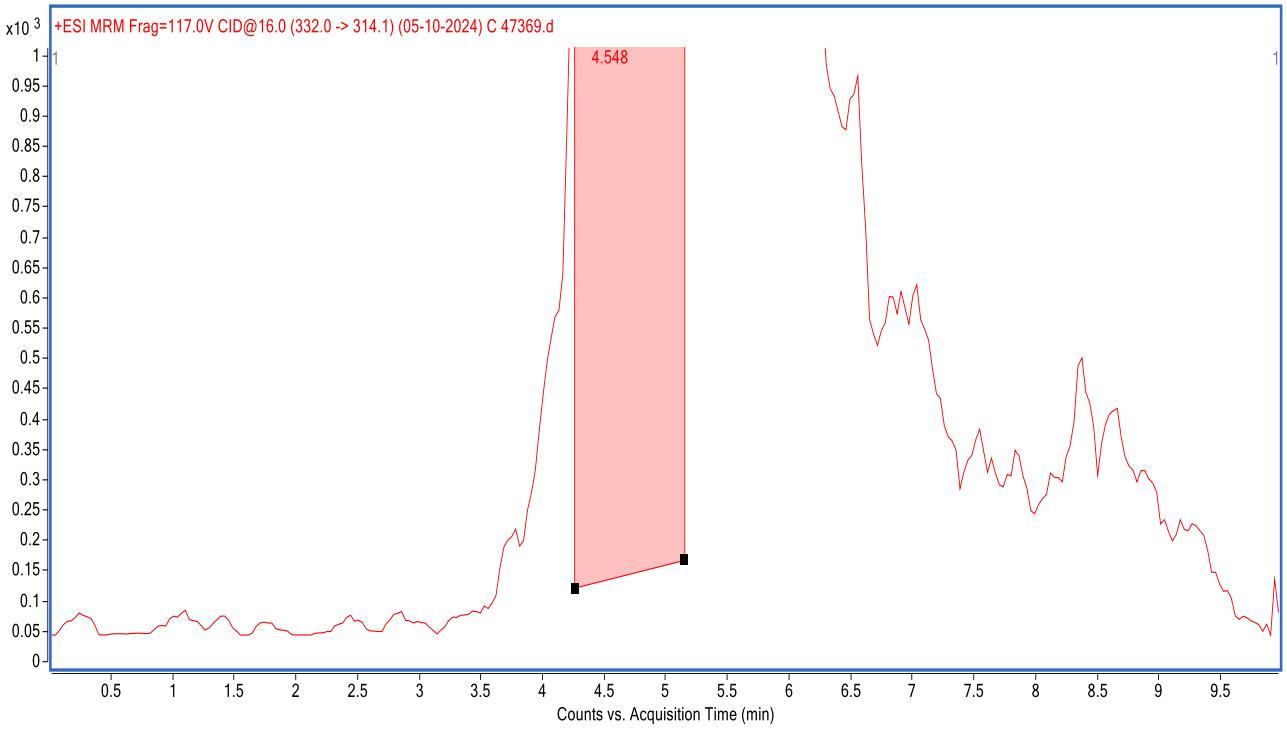


Fig 58: Ciprofloxacin Quantifier ion Zoomed view

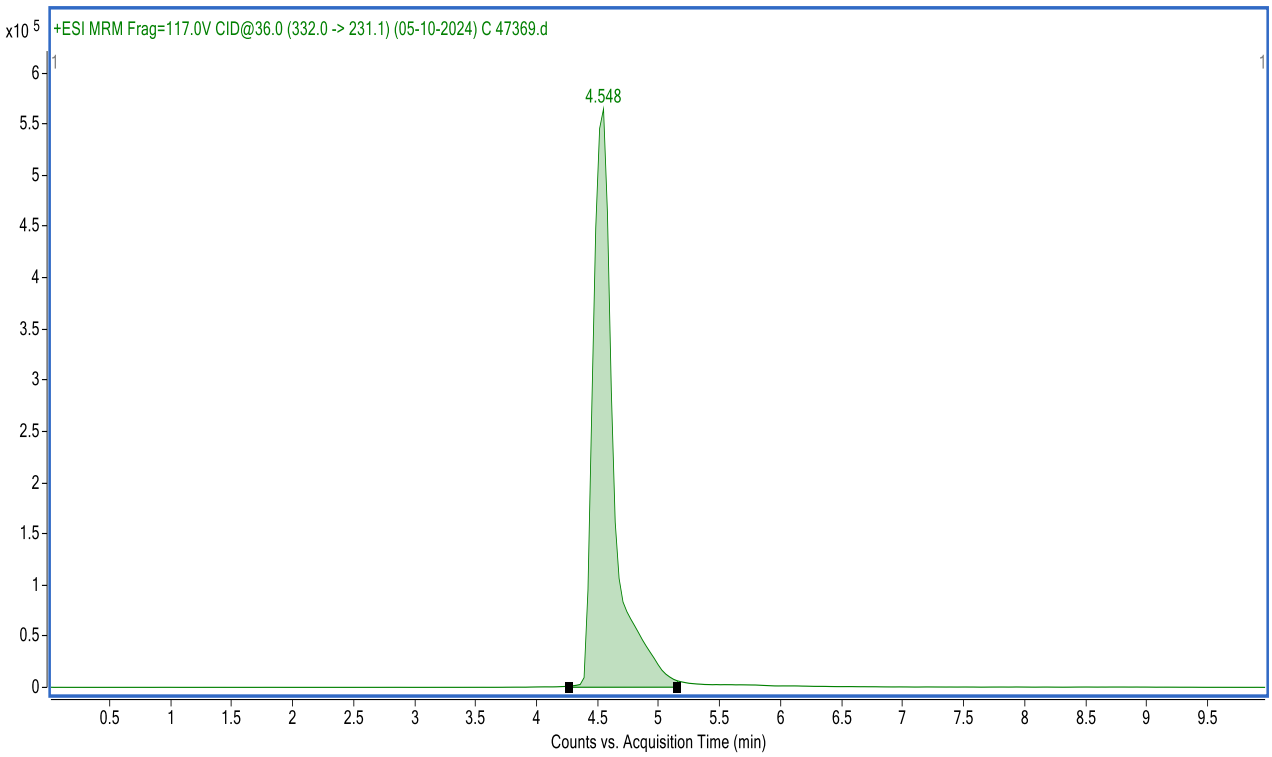


Fig 59: Ciprofloxacin Qualifier ion

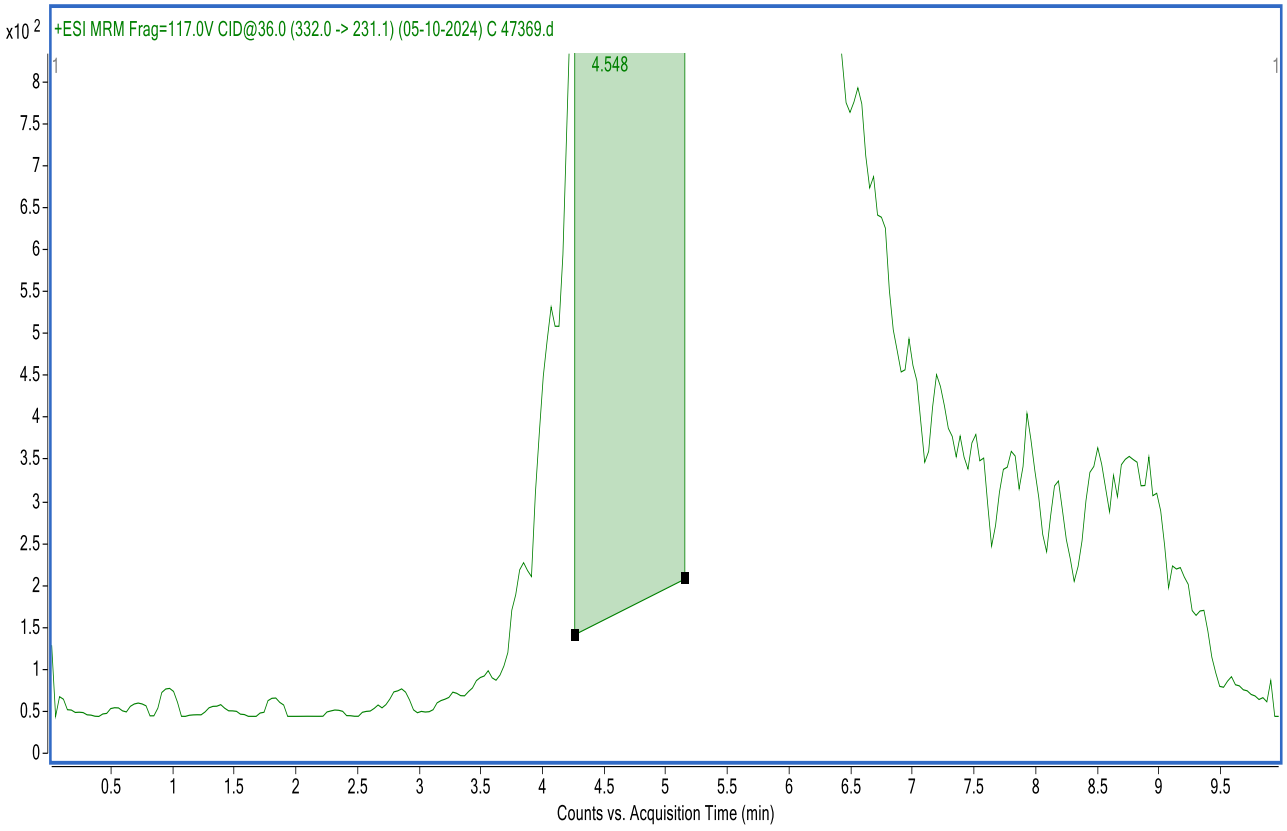


Fig 60: Ciprofloxacin Qualifier ion

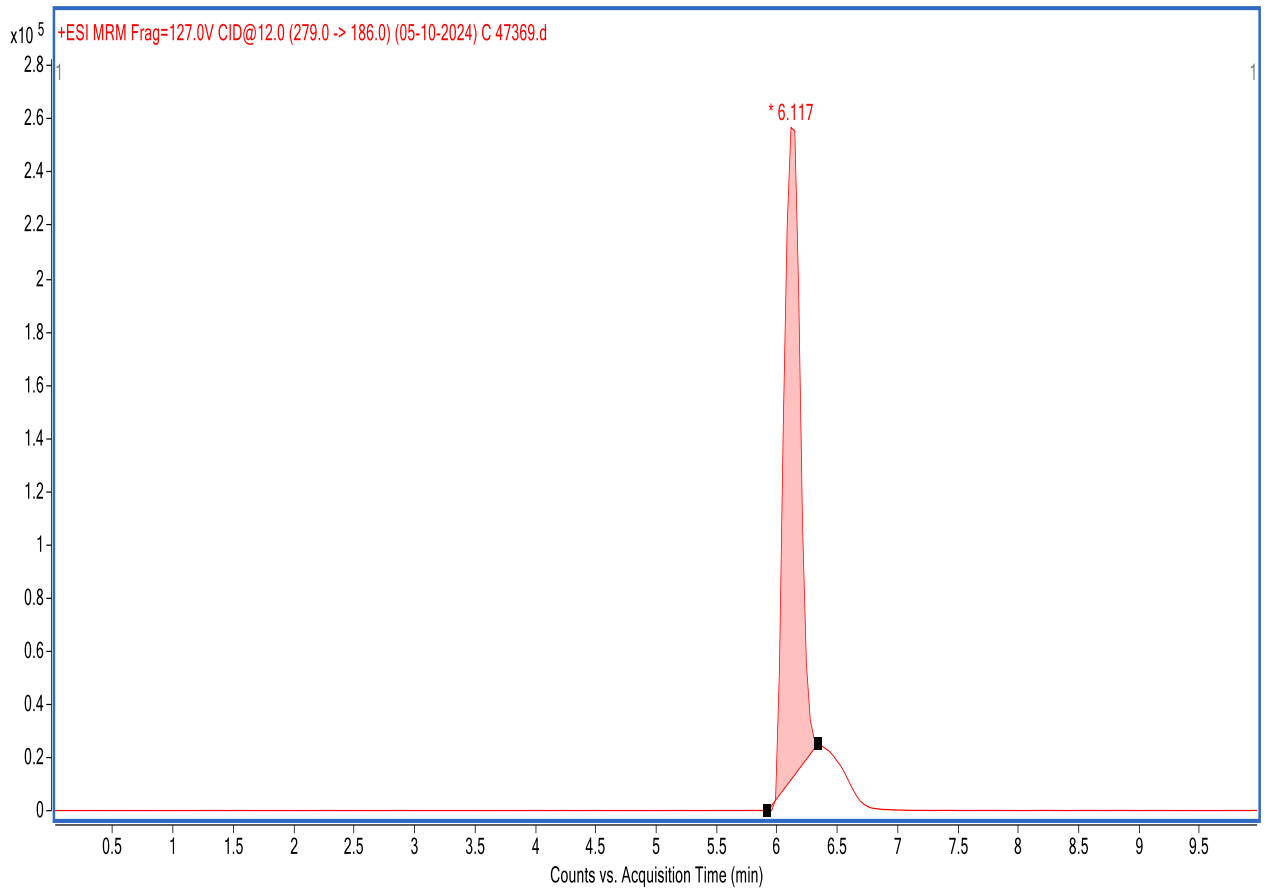


Fig 61: Sulfadimidin Quantifier ion

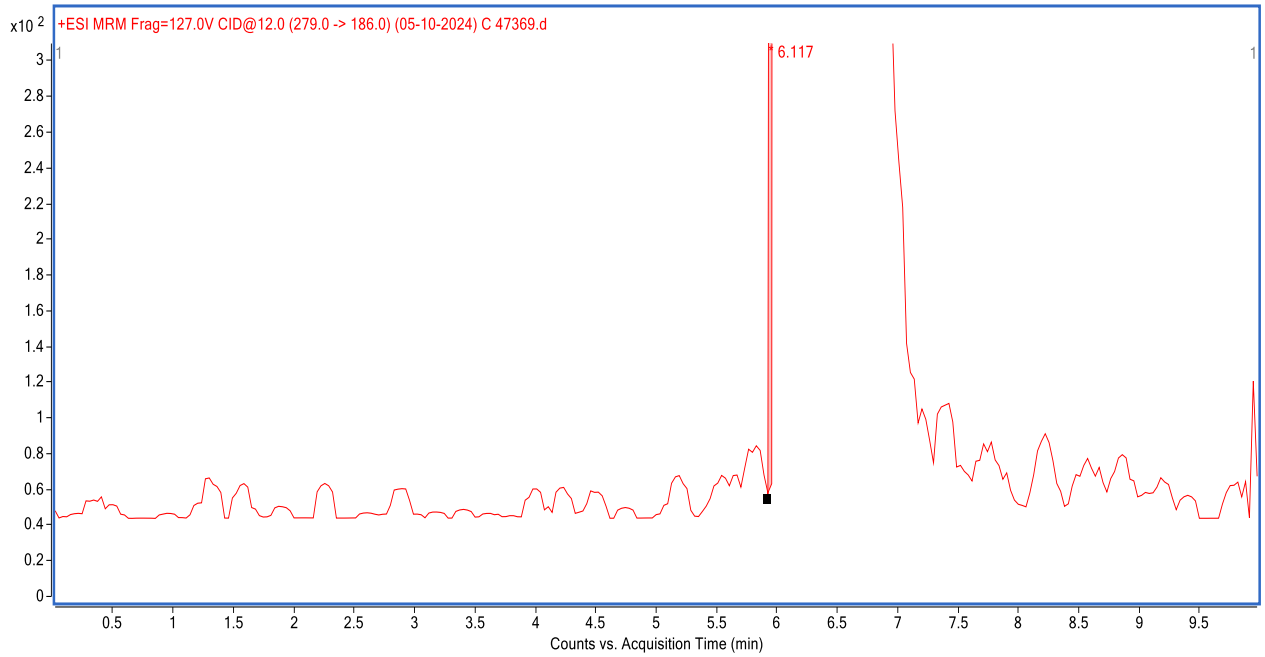


Fig 62: Sulfadimidin Quantifier ion Zoomed view

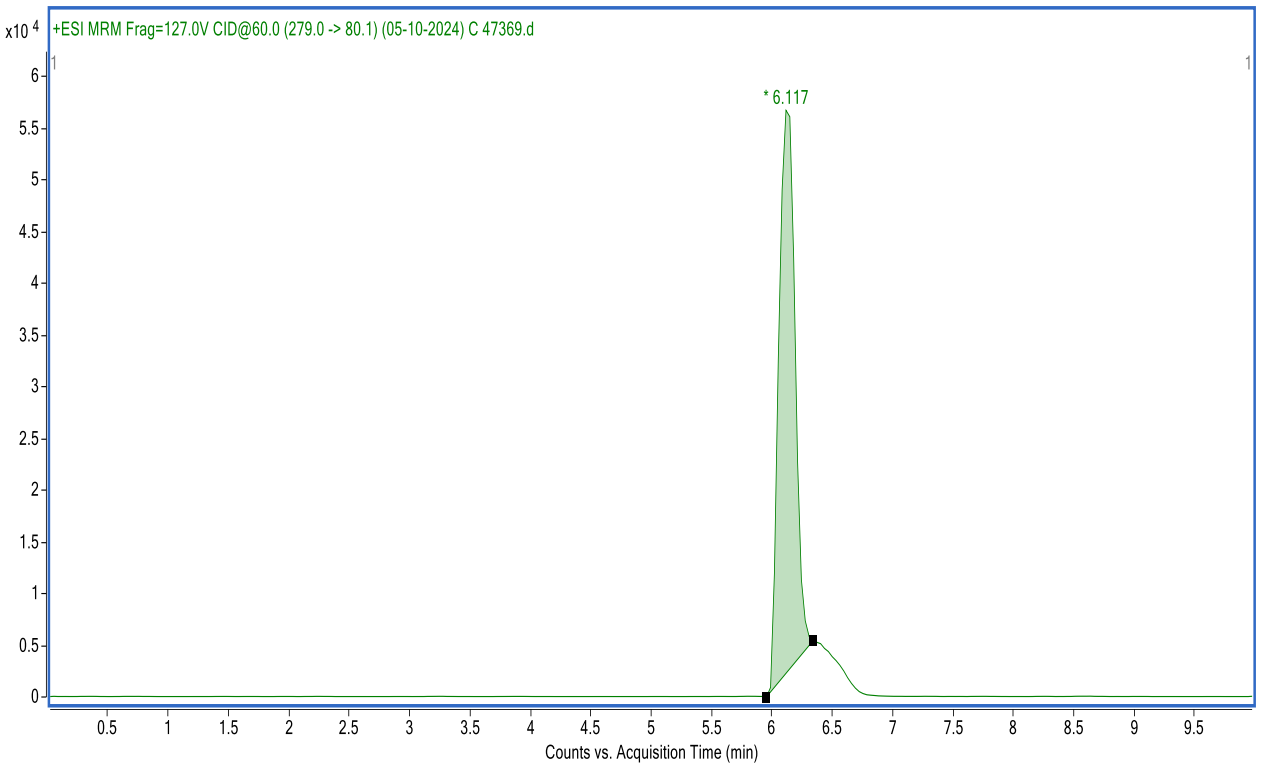


Fig 63: Sulfadimidin Qualifier ion

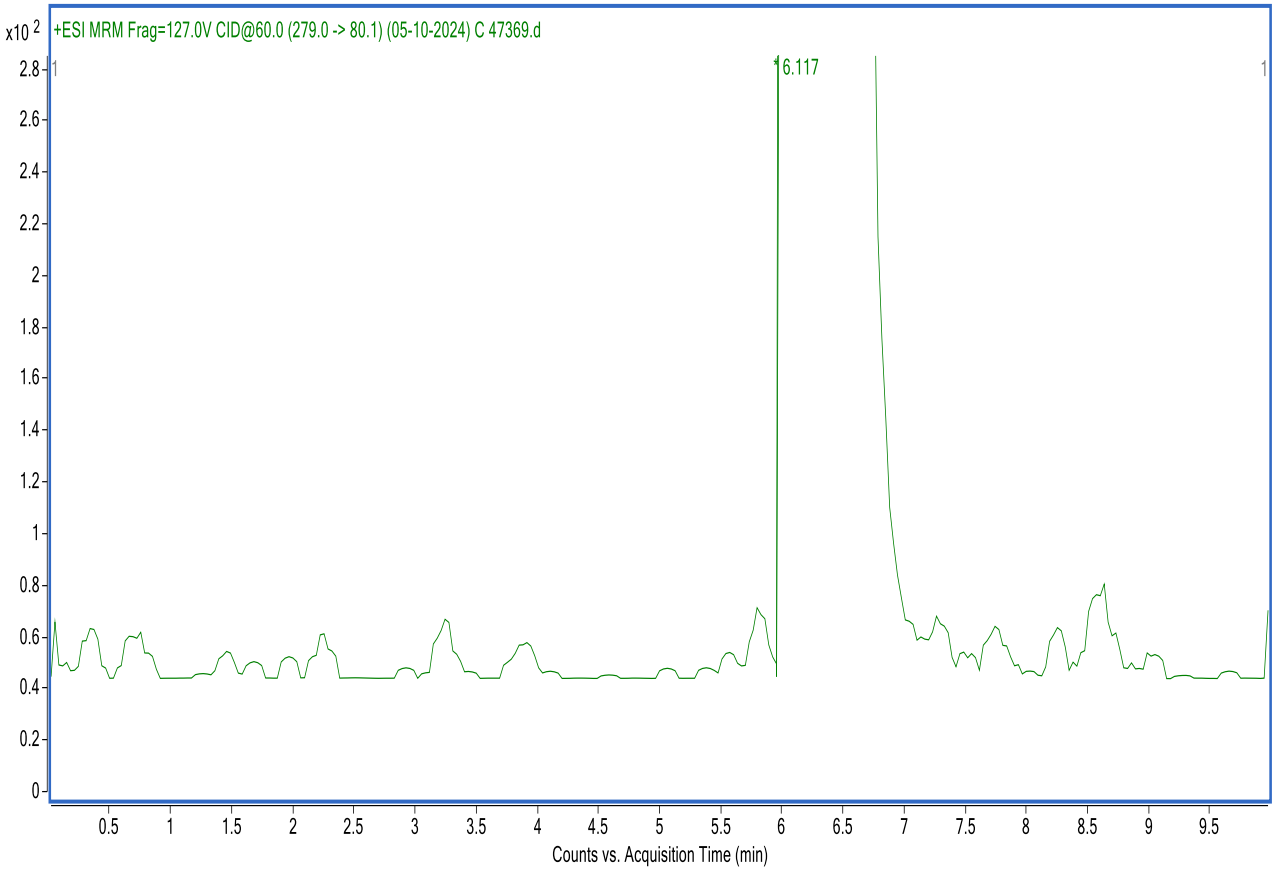


Fig 64: Sulfadimidin Qualifier ion Zoomed view

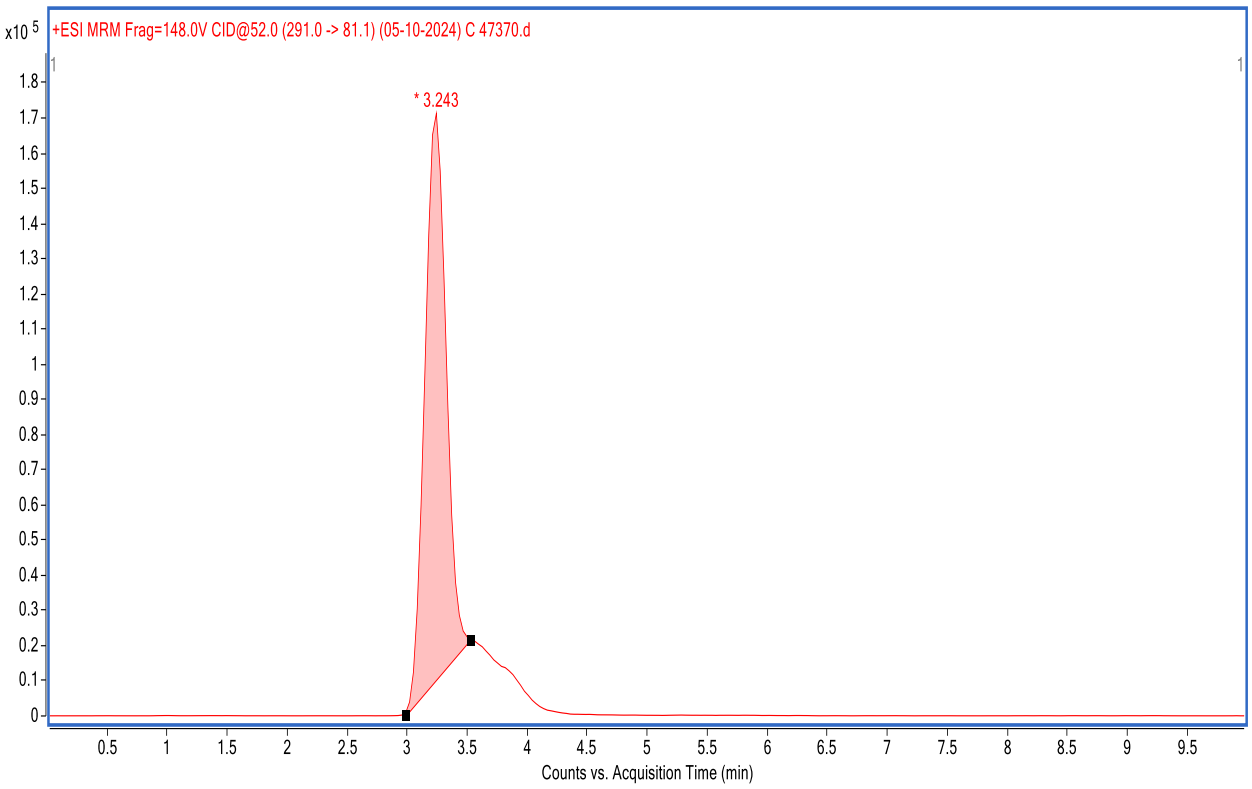


Fig 65: Trimethoprim Quantifier ion

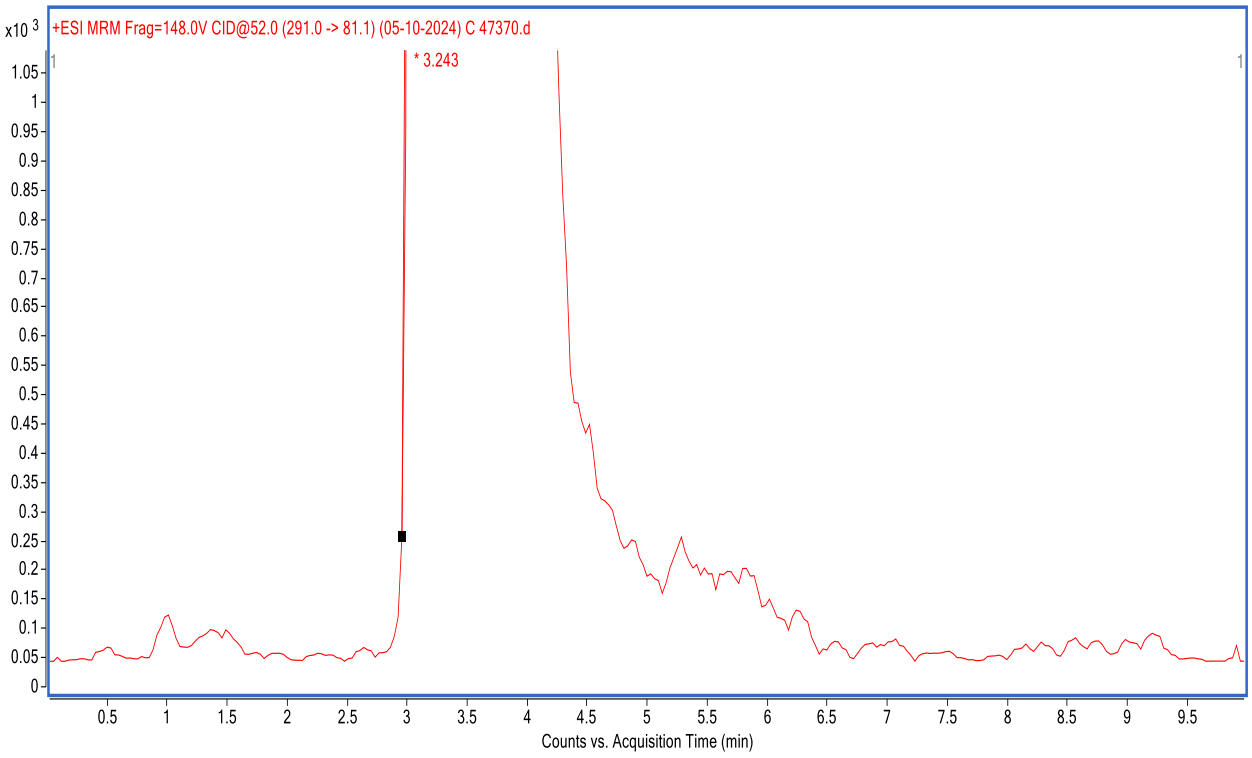


Fig 66: Trimethoprim Quantifier ion Zoomed view

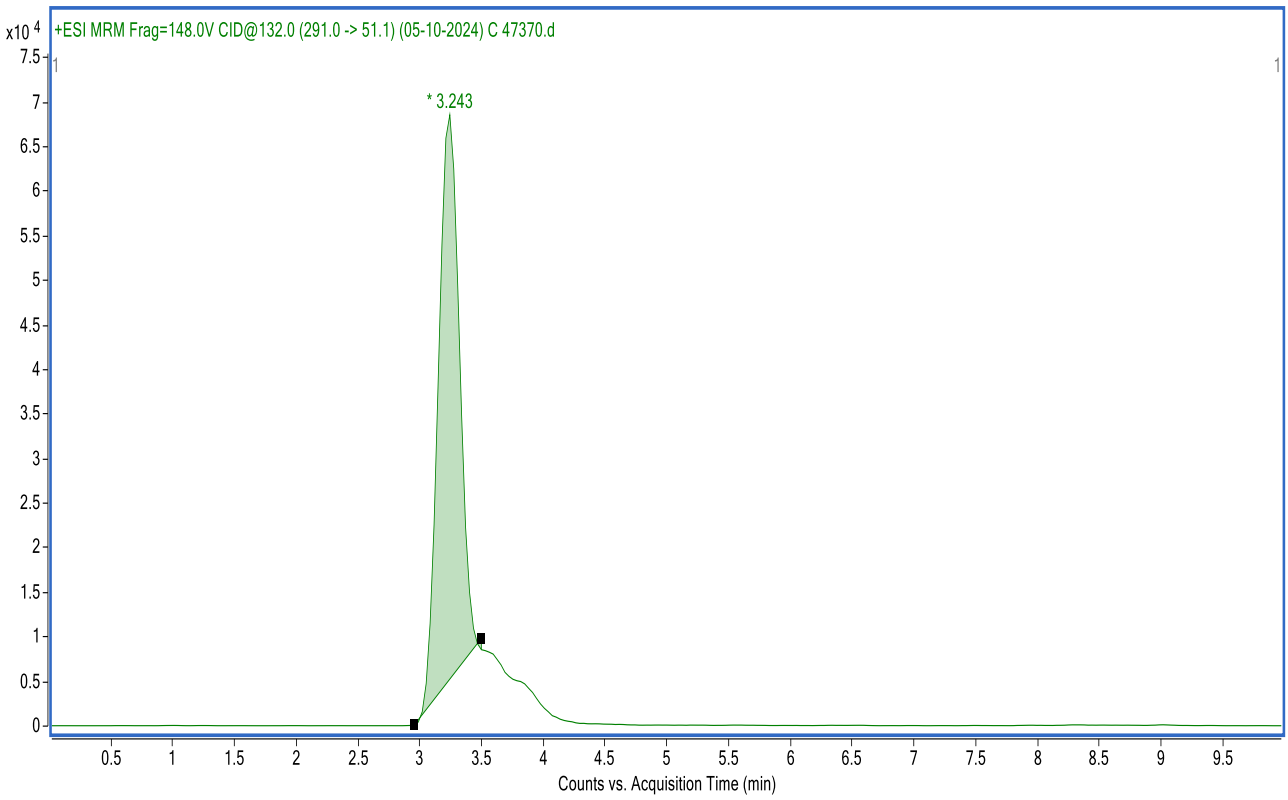


Fig 67: Trimethoprim Qualifier ion

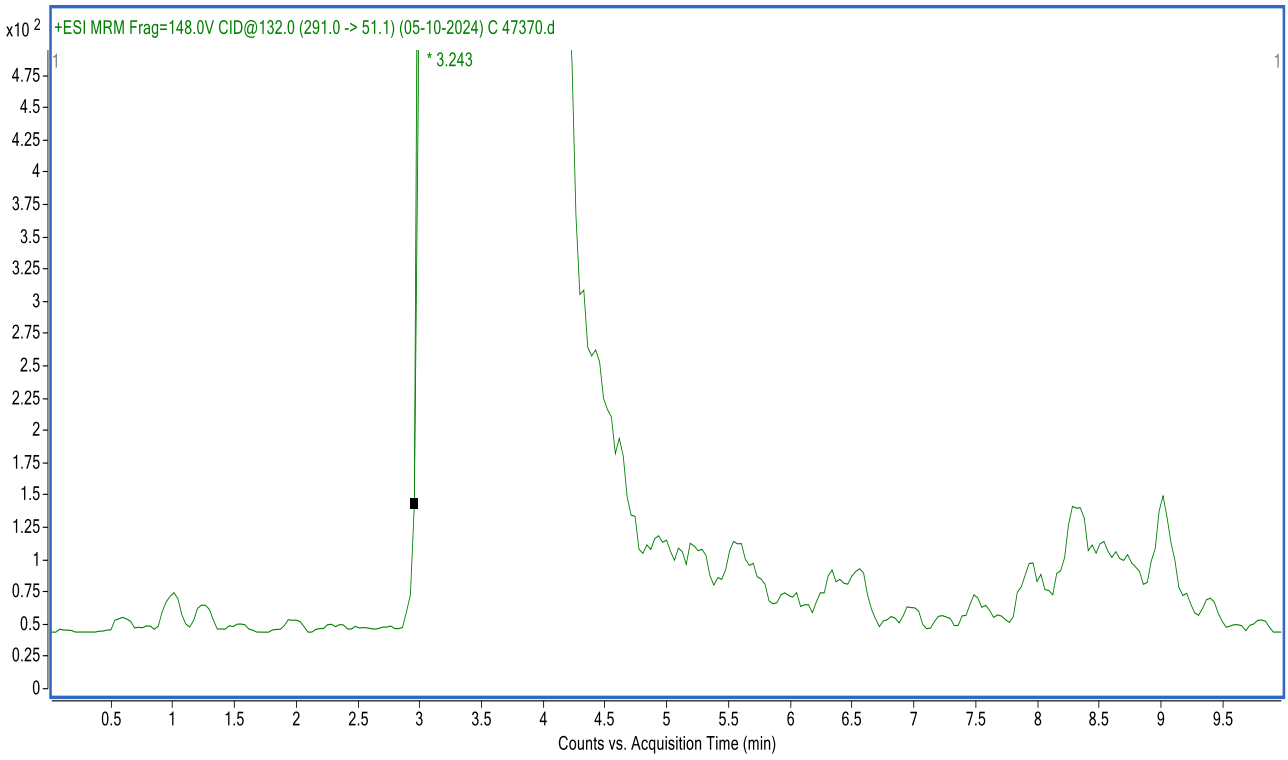


Fig 68: Trimethoprim Qualifier ion Zoomed view

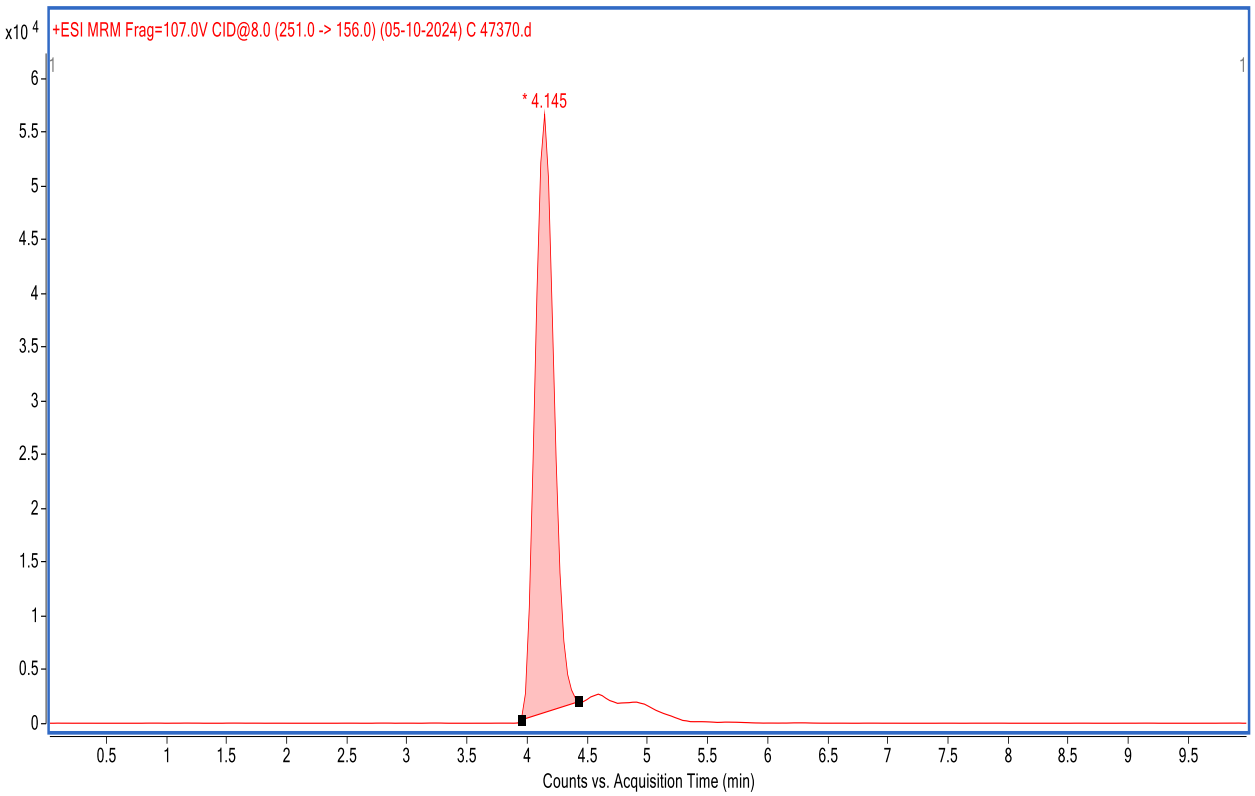


Fig 69: Sulfadiazin Quantifier

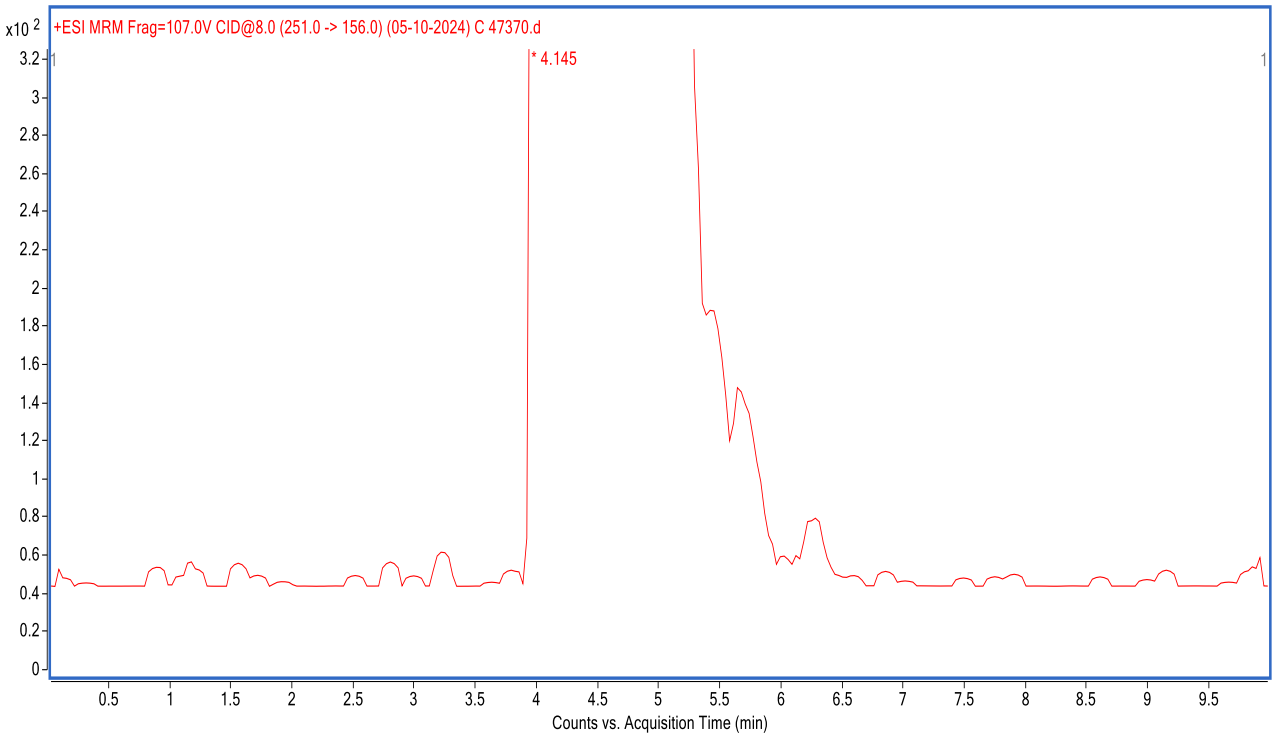


Fig 70: Sulfadiazin Quantifier Zoomed view

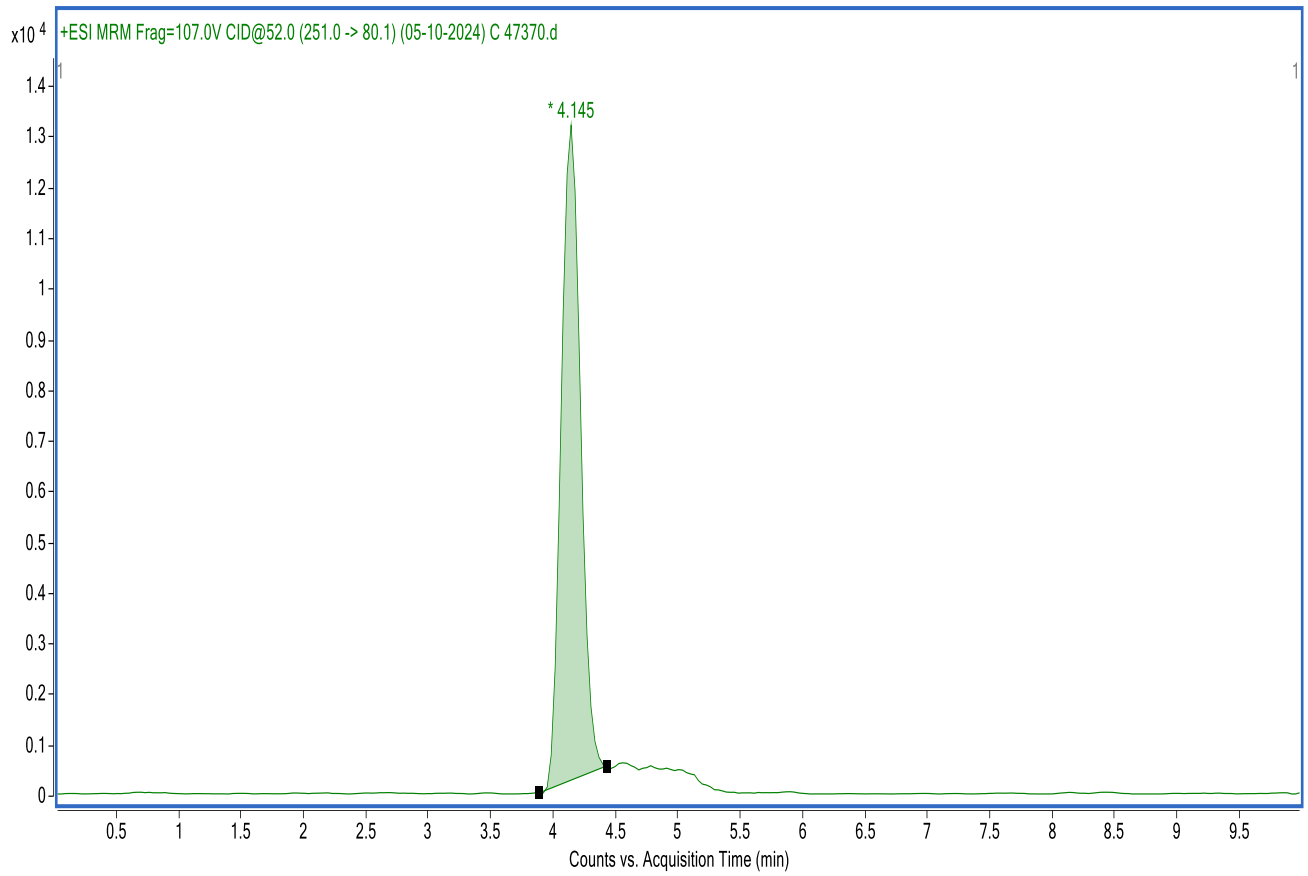


Fig 71: Sulfadiazin Qualifier ion

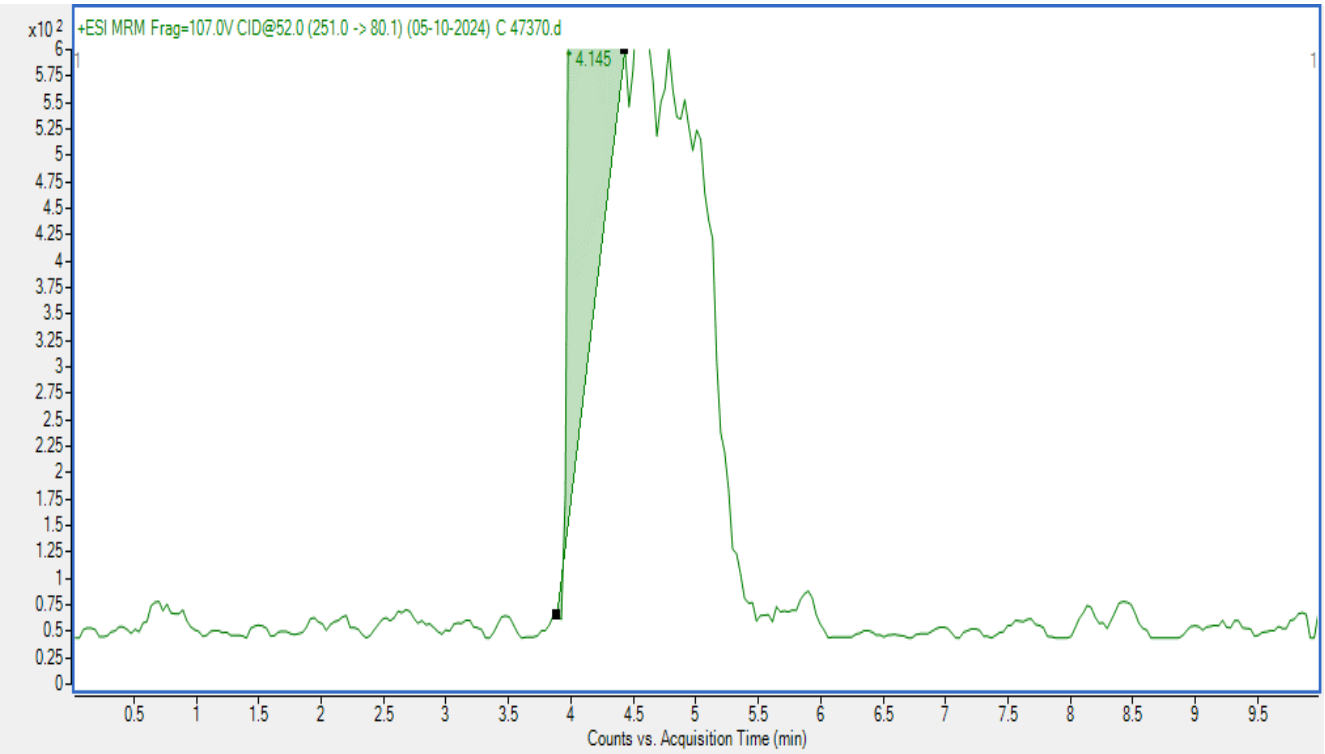


Fig 72: Sulfadiazin Qualifier ion Zoomed view

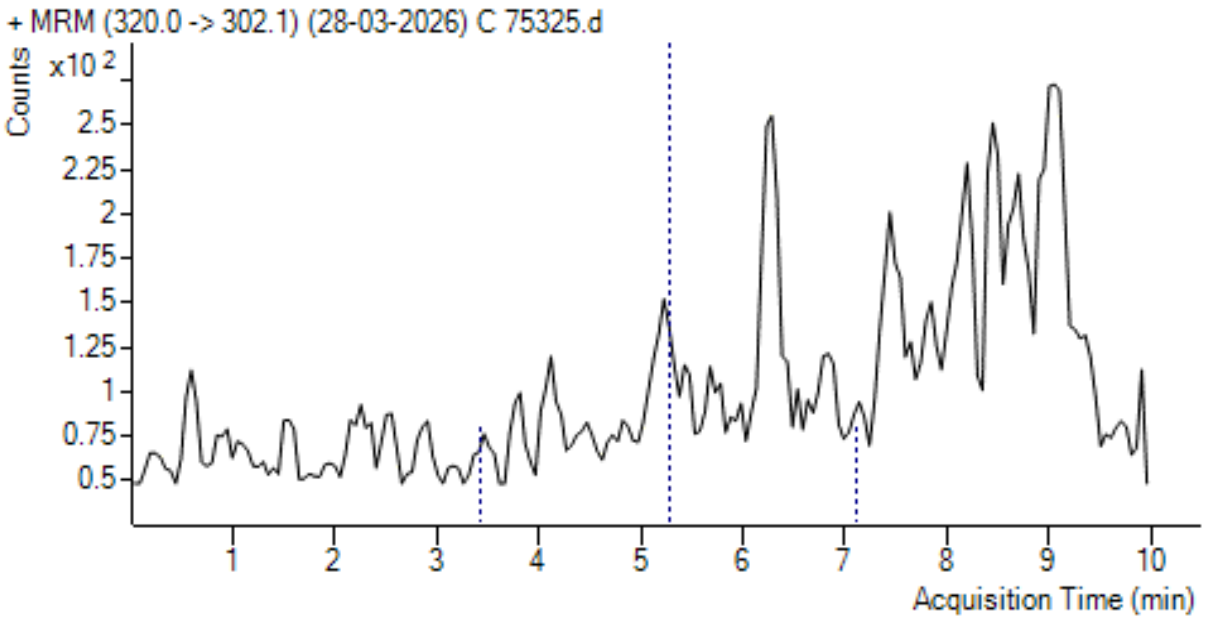


Fig: 73 Quantifier Chromatogram of Norfloxacin (Control of Shrimp Gut,soil and water –Munroe Island)

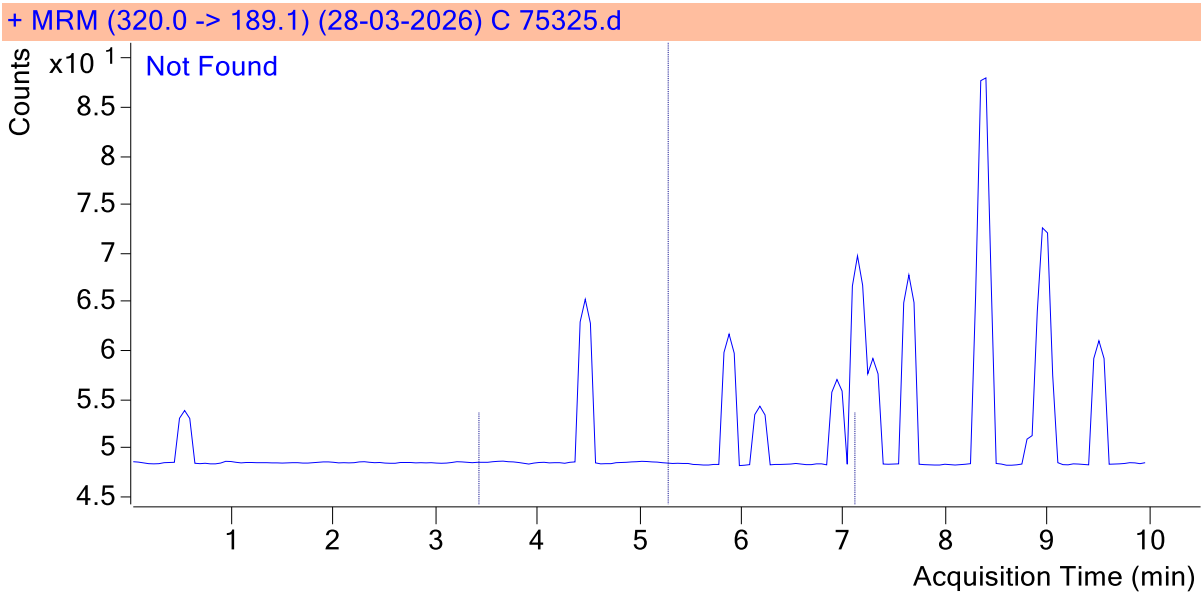


Fig 74:Qualifier Chromatogram of Norfloxacin - (Control of Shrimp Gut,soil and water –Munroe Island)

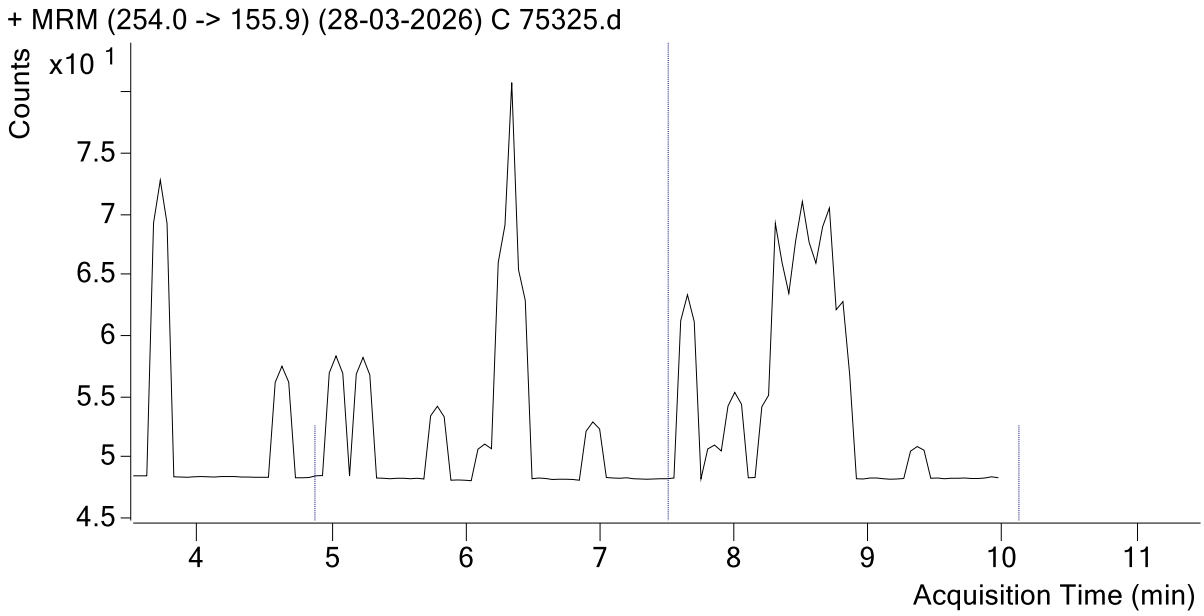


Fig 75:Quantifier Chromatogram of Sulfamethoxazole - (Control of Shrimp Gut,Soil and Water- Munroe Island)

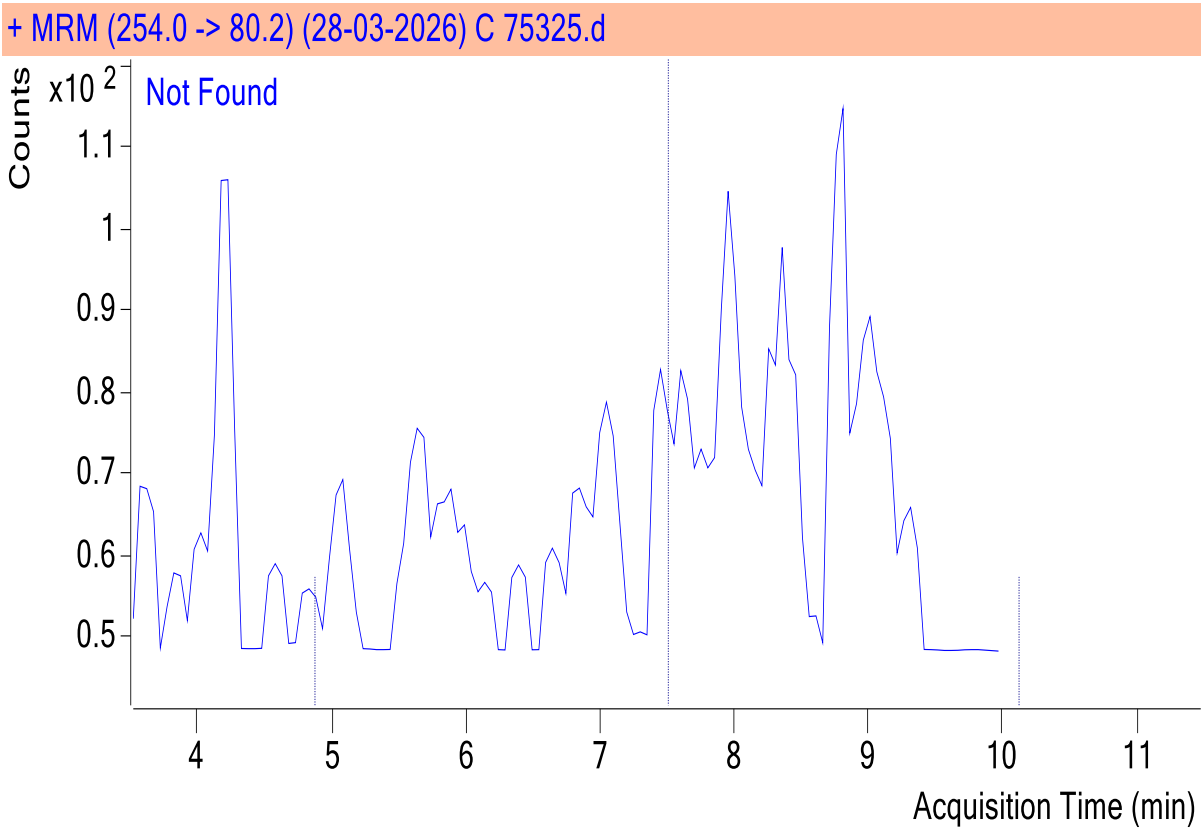


Fig 76 :Qualifier Chromatogram of Sulfamethoxazole - (Control of Shrimp Gut,Soil and Water-Munroe Island)

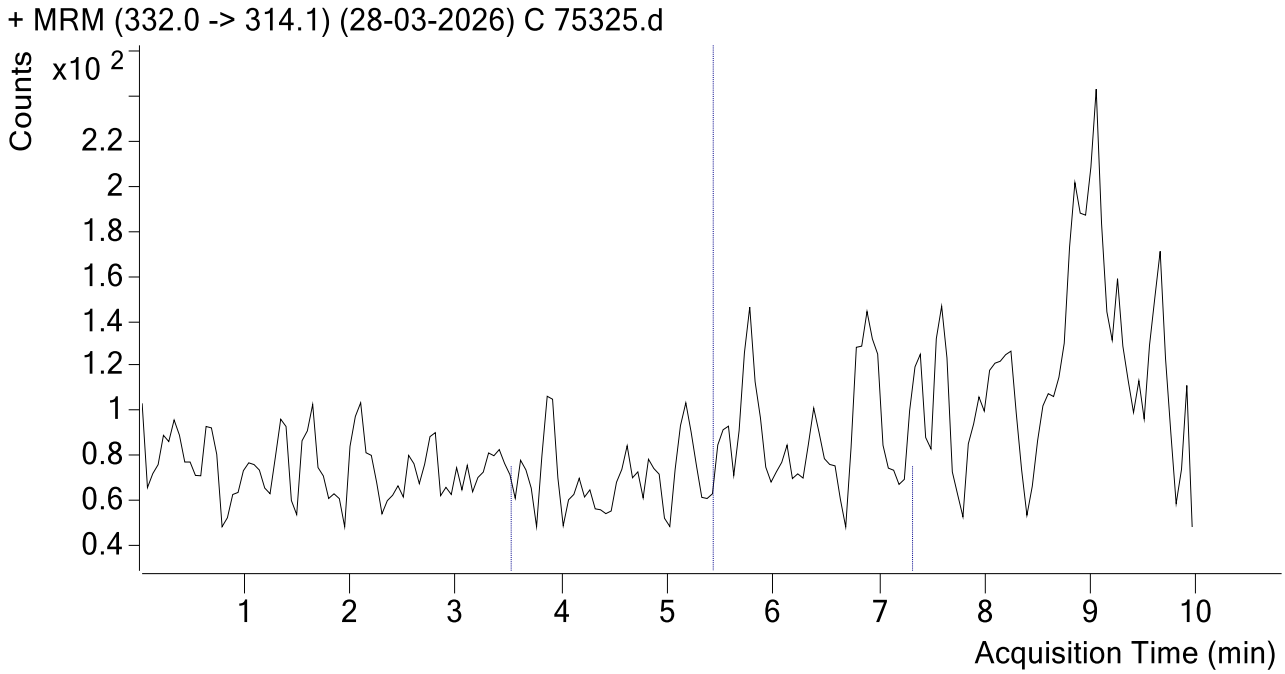


Fig 77:Quantifier Chromatogram of Ciprofloxacin- (Control of Shrimp Gut,Soil and Water – Anjuthengu)

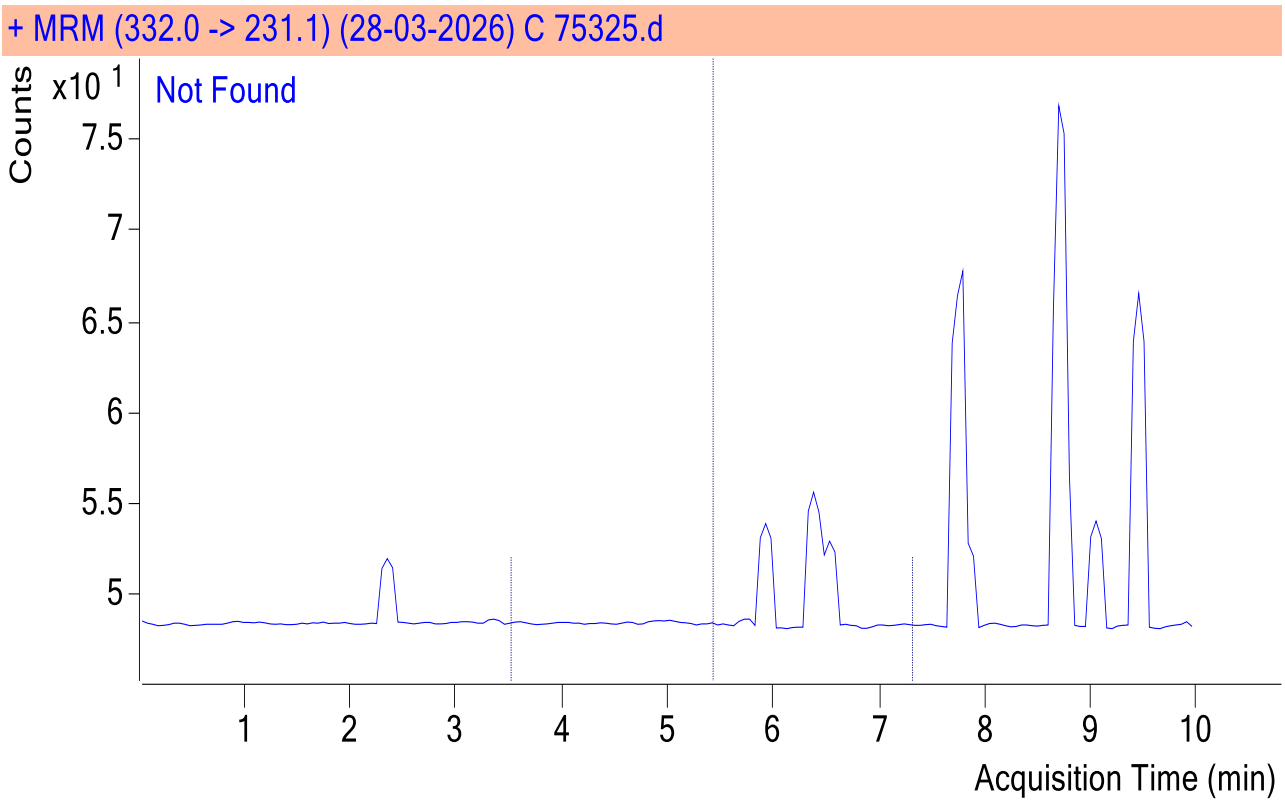


Fig 78:Qualifier Chromatogram of Ciprofloxacin- (Control of Shrimp Gut,Soil and Water – Anjuthengu)

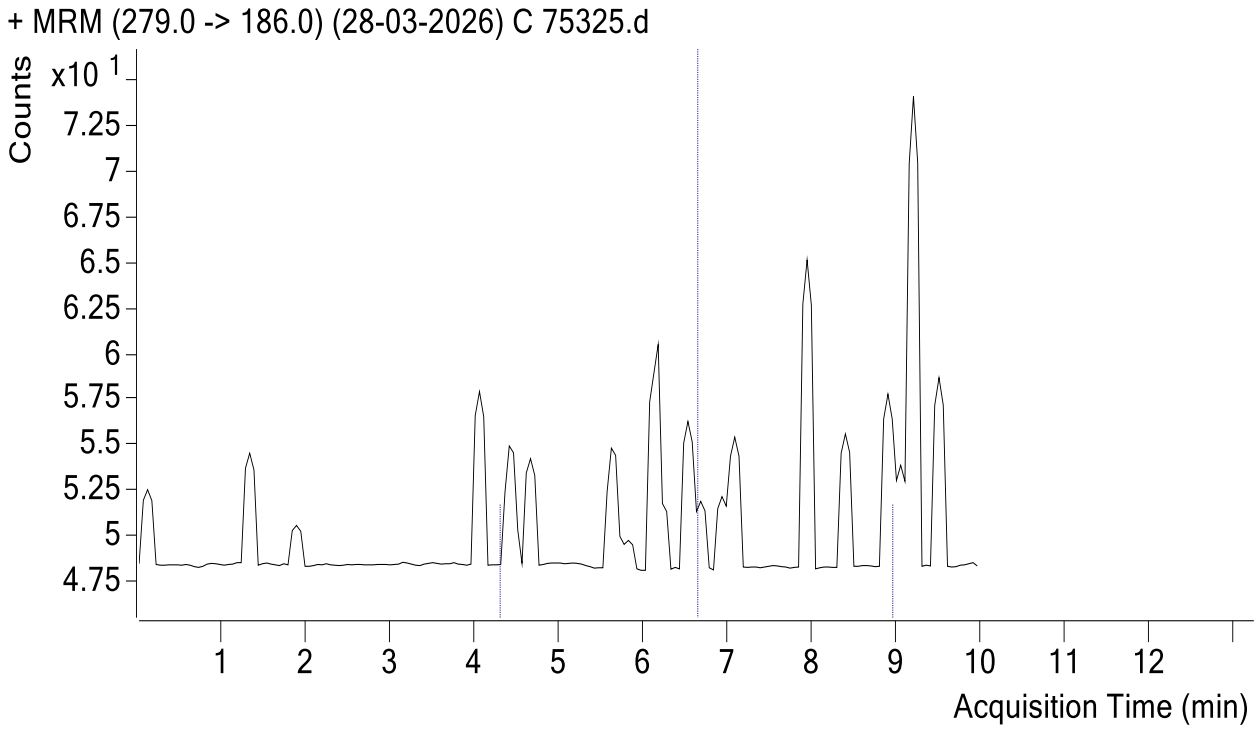


Fig 79:Quantifier Chromatogram of Sulfadimidin - (Control of Shrimp Gut,Soil and Water - Anjuthengu)

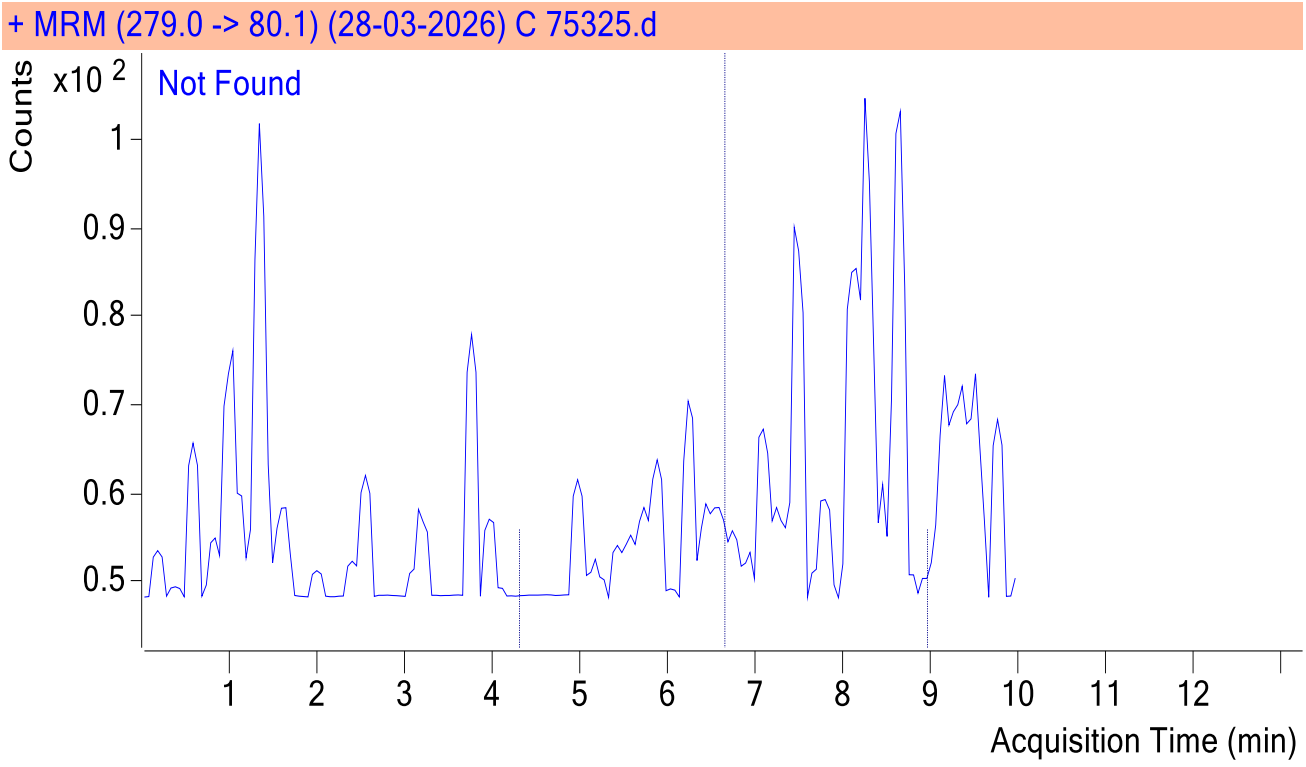


Fig 80: Qualifier Chromatogram of Sulfadimidin - (Control of Shrimp Gut,Soil and Water –Anjuthengu)

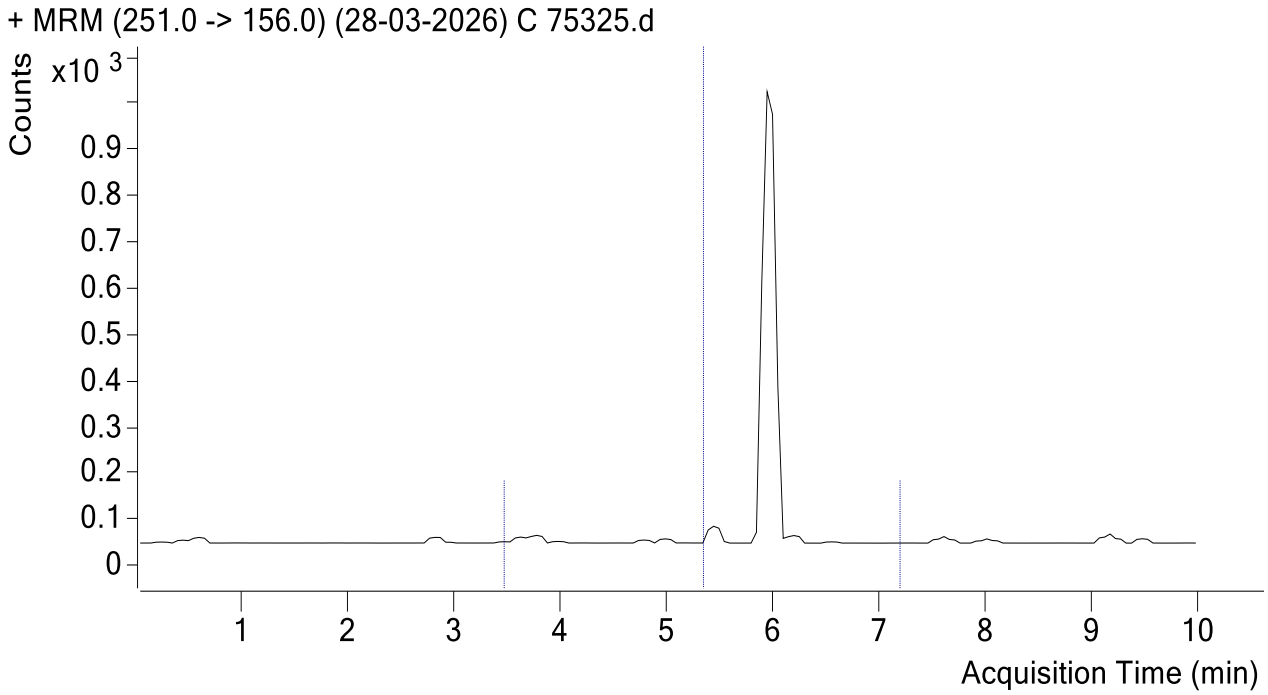


Fig 81 : Quantifier Chromatogram of Sulfadiazin - (Control of Shrimp Gut,Soil and Water – Neyyatinkara)

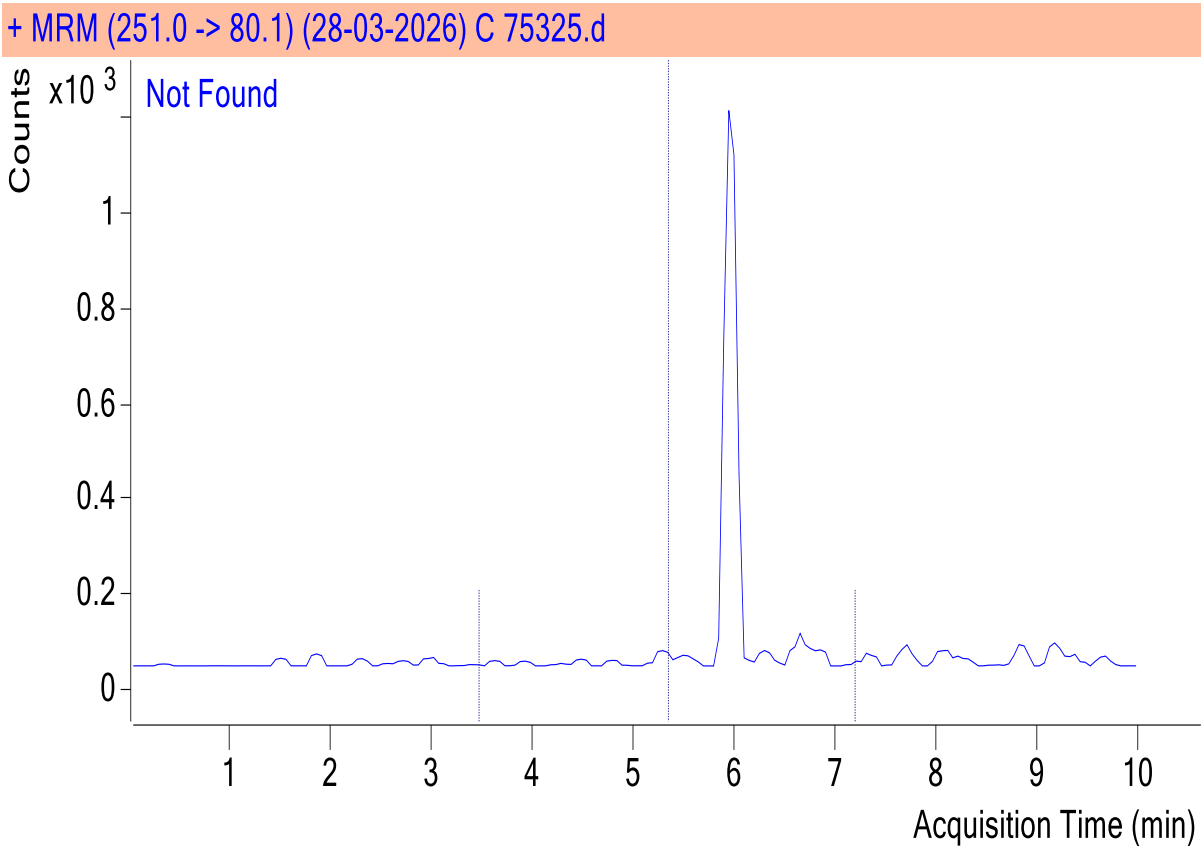


Fig 82 : Qualifier Chromatogram of Sulfadiazin - (Control of Shrimp Gut,Soil and Water – Neyyatinkara)

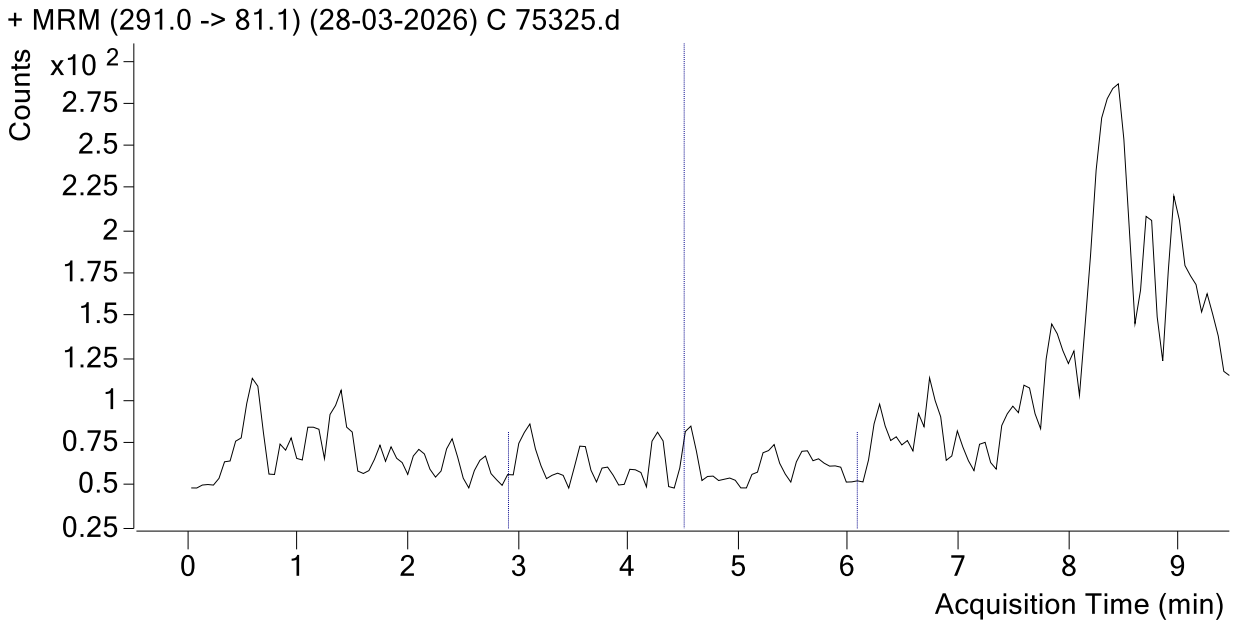


Fig 83 : Quantifier Chromatogram of Trimethoprim - (Control of Shrimp Gut,Soil and Water – Neyyatinkara)

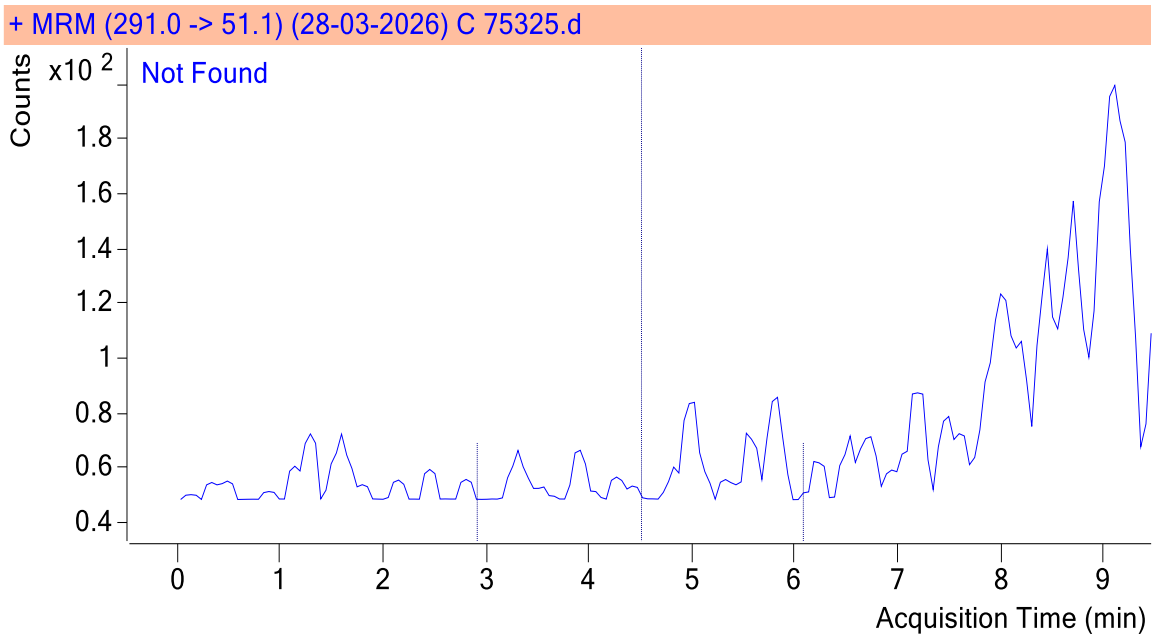


Fig 84: Qualifier Chromatogram of Trimethoprim (Control of Shrimp Gut,Soil and Water – Neyyatinkara)

4.Conclusions

A simple, cheap, fast, reliable and sensitive multiclass antibiotic residue analytical method was developed for the simultaneous determination of antimicrobial drugs (23 compounds from three different families) in shrimp gut, pond water and soil samples from the southern coastal areas of Kerala using simple liquid extraction with UHPLC-MS/MS. This method can detect antimicrobial drugs within a single run and was demonstrated to be an excellent tool for explicit identification of selected compounds using the retention time of quantification and confirmation ions. The results showed that the investigated southern coastal area of Kerala, shrimp cultivated farms in Munroe Island, Anjuthengu and Neyyatinkara contains the residues of norfloxacin, sulfamethoxazole, ciprofloxacin, sulfadimidin, sulfadiazin, and trimethoprim antibiotics residues in respective shrimp farms. For the convincingness of our work we examined all the samples with the same parameters and obtained the observation which were completely granting the entire result of the work. The antibiotic residues in food products were causing serious health problems in humans and animals. So being aware of these consequences, the shrimp farms should be well monitored and farmers should be well consciousness for antibiotic application .

5.Funding Declaration

No external funding was received during this work.

6.Significance of the present study

Shrimps are one of the top internationally traded seafood commodities across the globe with the majority of production coming from aquaculture. The most important challenges facing the shrimp farms were antimicrobial resistance due to the overuse of antibiotics in shrimp farms. Some shrimp farms are of major concern as a source for AMR since traditionally antimicrobial drugs are used in prophylactic and therapeutic purposes. Antimicrobial resistance is a global public health threat that occurs when bacteria, viruses, fungi and parasites evolve and stop responding to medicine. Most of the shrimp production occurs in Asia. In our study we clearly observed the presence of antibiotic residues in shrimp farms in Southern coastal areas of Kerala .

7.Conflict of Interests

The authors declare no competing interests.

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