

## **Review of Analytical Methodologies for the Determination of Metronidazole and Trimethoprim in Environmental Samples**

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### **Abstract**

Antibiotics are essential for treating infectious diseases, but their overuse and adverse effects are raising concerns about global public health. The pervasiveness of antibiotic contamination in aquatic environments has drawn increased attention in recent years. The primary concern regarding the release of antibiotics into the environment is the potential for microorganisms to become resistant to antibiotics. This review article summarizes the analytical methods used to determine the presence of trimethoprim and metronidazole in various environmental samples. These antibiotics have traditionally been analyzed using tandem mass spectrometry or high-performance liquid chromatography coupled to mass spectrometry; fluorescence or ultraviolet detection has been used less frequently. An essential step before liquid chromatography analysis is preparing the sample for extraction and analysis. This helps to eliminate interferences, stop the matrix effect, and pre-concentrate the target analytes. Consequently, the purpose of this work is to provide an overview of the most widely used techniques for the determination of metronidazole and trimethoprim in environmental samples.

**Keywords:** Analytical methodologies; metronidazole and trimethoprim; Environmental samples

### **Introduction**

Antibiotics are essential for treating a wide range of infectious diseases, including gonorrhea, urinary tract infections, and tuberculosis, which can be fatal to humans. They belong to a powerful class of drugs that are widely used to either kill or inhibit the growth of bacteria. However, because of their adverse effects and indiscriminate prescribing, antibiotics are becoming an increasingly serious global public health concern. Antibiotics work through a variety of mechanisms, one of which is the inhibition of peptidoglycan and nucleic acid synthesis. This process inhibits cell division and ultimately results in cell death. Drug-resistant bacteria are becoming more widely recognized, and the lack of new antibiotic development combined with their potential negative effects on health could pose challenges in the future [1]. Microorganisms can be killed or their growth stopped by antibiotics, which are the strongest drugs [2].

Recently, many distinct kinds of newly discovered contaminants in water systems have been identified as novel environmental risks that require appropriate

management [3]. Wastewater pollution affects community water supplies and can contain heavy metals [4] and pharmaceutically active substances that are harmful to health. Many pharmaceutical substances have been detected in surface water, groundwater, and wastewater treatment plant effluents because they have been utilized since the 1950s as a result of the rapid population growth and advancement of medical science [5]. Pharmaceuticals and pharmaceutically active compounds are currently categorized as emerging environmental contaminants due to their unavoidable rise in use and growing presence in a range of environmental compartments [6]. Environmentalists are having issues with new pollutants found in surface water bodies across the globe, such as phenols [7], polycyclic aromatic hydrocarbons [8], and pharmaceuticals and personal care products.

Pharmaceuticals and personal care products (PPCPs) are highly consumed and can enter the water system either directly or indirectly through human activities. As a result, they can end up in surface and groundwater at concentrations ranging from ng/L to mg/L. It has been demonstrated that prolonged exposure to low, subtoxic concentrations of several PPCPs can have negative impacts on humans and ecosystems [9–11]. The toxicity, effects, and behaviors of many PPCPs are unknown, and only a small proportion are regularly monitored in the environment. As a result, many PPCPs are uncontrolled [12]. Over the past few decades, numerous PPCPs have been found in aquatic environments all over the world [13]. Because of their widespread use, PPCPs have the potential to enter surface and groundwater through anthropogenic processes such as animal husbandry, fertilizer, sewage discharge, and landfill leachate [13].

The global health community is facing a challenge due to the widespread reporting of antibiotic-resistant bacteria growing in the environment in recent years [14–16]. One major source of antibiotics that find their way into natural rivers is wastewater treatment plant (WWTP) discharges [17–20]. The receiving water body experiences an instantaneous effect from these releases [21,22]. It is now crucial to create advanced analytical methods for wastewater antibiotic monitoring [23,24], to achieve several objectives: Conducting appropriate environmental risk assessments, assessing potential problems, assessing the effectiveness of WWTPs in eliminating them, monitoring the success of tertiary systems used to improve their removal, estimating the consumption of a particular population through wastewater analysis [25–30]. The requirement for the use of extremely sensitive analytical methods and the diverse physicochemical properties of the antibiotic family present an analytical challenge in determining the antibiotics.

Antibiotics are crucial in the treatment of numerous infectious diseases, including gonorrhea, urinary tract infections, and tuberculosis, which can be major causes of mortality [31]. They are the strongest medications and are widely used to either eradicate or inhibit the growth of bacteria [32,33]. However, because of their adverse effects and inappropriate antimicrobial prescription practices, antibiotics are becoming a global public health concern [34]. Antibiotics work in a variety of ways,

such as by blocking the synthesis of peptidoglycan, nucleic acids, and replication. Antibiotics are a type of pharmaceutical that is frequently used to treat microbial infections, which are thought to be a significant class of organic pollutants in water with a variety of adverse environmental impacts [35–37]. Particularly prevalent pharmaceutical pollutants that are extremely dangerous for both human health and the environment are antibiotics [38].

The antibiotic metronidazole (MET), one of the most often used medications in the world, treats infectious diseases caused by anaerobic bacteria and protozoa <sup>(5)</sup>. MET has been widely utilized for the diagnosis and surveillance of protozoal disorders, such as trichomoniasis and giardiasis, due to its antibacterial and anti-inflammatory characteristics [39]. However, considering its genotoxic, carcinogenic, and mutagenic adverse effects, prolonged consumption of this chemical may seriously harm human health [40]. The persistent presence of MET and its metabolites in the environment, coupled with their biological characteristics, could potentially have substantial, long-term impacts on ecosystem stability, given their mutagenic, carcinogenic, and toxic characteristics. Strong and trustworthy examination procedures are needed in conjunction with confirmatory testing that can identify MET residues at a low level to guarantee the safety of food for human consumption and to safeguard the environment. Thus, developing techniques for MET determination in biological products and environmental samples is one of the most important analytical tasks in environmental [41].

Because they are simple and reasonably priced, spectrophotometric techniques can be useful for identifying environmental samples [42,43]. Furthermore, ionization mass spectrometry has been applied to analyze a broad range of analytes, such as minor medicinal substances [44]. In the study by Han and co-workers, the Raman spectra of metronidazole have been obtained from environmental samples such as soil, tap water, lakes, and swamp waters. The limit of detection for MET in these samples is 10 µg/mL, as well as from 29 ultra-pure water samples [45].

Liquid chromatography-ion trap mass spectrometry (LC-MS/MS) was also used to measure MET in water and sediment samples. Solid phase extraction (SPE) was used to extract MET from waters on Strata XC cartridges. SPE was used to extract MET from sediments and fish tissues. The greatest concentrations were found in tissue, sediment, and water, respectively, at 1.5 ng/ g, 12.0 ng/ g, and 136.2 ng/ L. The findings verified the suitability of these techniques for concurrently analyzing fish tissues, sediments, and waters for the presence of MET [41].

Rapid extraction techniques in conjunction with short-column high-performance liquid chromatography with diode-array detection (HPLC-DAD) enabled the identification of five important antibiotics, including MET, in extremely complex wastewater samples. The samples were separated on an ODS column (7 cm) in less than 4 minutes after being pre-concentrated on Bond Elut-ENV cartridges. Recoveries between 69.6% and 120.3% with relative standard deviations of less than 11.0% show how well this technique works. The findings explained why samples

with one or more uncalibrated components had the second-order advantage for analytes and showed a strong correlation with wastewater samples [46].

The MET compound is categorized as being highly soluble in water and poorly biodegradable. Because only small amounts of the catalyst are required for the process, heterogeneous photocatalysis is the most promoted method of purifying water. This study set out to determine the ideal circumstances for the photocatalytic elimination of MET from aquatic specimens. The impact of mass, irradiance intensity, and catalyst type on metronidazole removal efficiency was ascertained. This study identified the transformation products that were produced and assessed the compound's degree of mineralization. Depending on the type of catalyst, metronidazole removal efficiency ranged from 50 to 95 percent. The combination of 12.5 g TiO<sub>2</sub>-P25 + PbS (1:1; v/v) yielded the highest MET conversion (95%) when the process was run for 60 minutes at 1000 W m<sup>-2</sup>. Untargeted analysis revealed the identification of four MET degradation products, which resulted from metronidazole rearrangement and C-C bond breaking [47].

Two straightforward, quick, inexpensive, and reliable chromatographic methods were presented and approved for use in the quantitative assessment of MET residues in samples of production wastewater. Before analysis, the samples were prepared on bond Elut C18 packs utilizing a solid-phase extraction technique. TLC densitometric determination at 278 nm was the first method used. The mobile phase is a 75:25 v/v acetonitrile/phosphate buffer mixture with a pH of 3. Ophosphoric acid was used to adjust the pH, and the flow rate was adjusted to 1.5 mL/min. The ICH guidelines have been followed in the confirmation of the previously mentioned techniques. The procedures outlined were utilized to precisely evaluate the drug residues examined in real industrial wastewater samples to ensure that they are absent so that they can be recycled and applied to other uses, such as irrigation [48].

Trimethoprim (TMP) is an antibiotic that is only utilized in bladder treatment. Aminopyrimidines belong to the trimethoprim class of chemicals. It has an inhibitor of sulfonamide dihydrofolate reductase, which is a regularly prescribed synergistic antibacterial drug primarily used for treating and preventing infections of the urinary tract [49]. TMP was first made accessible in 1969 when it was coupled with sulphamethoxazole (Co-trimoxazole). Since 1980, it has been sold in the US and Scandinavia [50]. TMP is one of the most widely used antibiotics. Because of its persistent behaviors, its presence in aquatic environments has raised serious concerns in recent years [51].

Two techniques (CZE-MS and CZE-MS/MS) were created for the multi-residue identification of TMP, a potentiator whose contents in animal edible tissues are governed by EU Council Regulation No. 2377/90. To maximize the electrospray conditions, experimental designs were used. The trueness and performance characteristics of the suggested procedures have been compared. All of the detected and quantified limits were below the maximum residue limits. This allowed for the

monitoring of these compounds in animal-derived foods and environmental samples, enabling the low  $\mu\text{g/L}$  range determination of TMP [52].

Using SPE, silica cartridge cleanup, and sensitive liquid chromatography–electrospray tandem mass spectrometry, a method was developed for 16 sulfonamides and trimethoprim in different water matrices. With mean recoveries ranging from 62–102% across all matrices under study, 17 analytes had method detection limits of 20–200  $\text{pg/L}$  for influent, 16–120  $\text{pg/L}$  for effluent, and 8.0–60  $\text{pg/L}$  for river water. Residual TMP was analyzed using this method in wastewater and river samples from Japan [53].

A highly selective and sensitive method has been developed for the simultaneous detection of TMP, fluoroquinolones, sulfonamides, and macrolides in wastewater and river water. Samples were analyzed using reversed-phase liquid chromatography in conjunction with electrospray ionization tandem mass spectrometry, after being enriched using solid-phase extraction. For quantification, a number of stand-in standards were employed. In every matrix that was studied, the total recoveries of each antimicrobial ranged from 49 to 133%, with an RSD of 1 to 18%. Achieved detection limits in the low  $\text{ng/L}$  range. The technique was effectively used to analyze raw municipal wastewater, wastewater effluents, and waterways of rivers [54].

Sandra and colleagues' study outlines a procedure for separating and measuring an antibiotic mixture that was taken out of water samples that had been tampered with. SPE is used to separate the analytes from the water, and TLC is used to analyze the extract. Using chloroform–methanol (89: 11), as the mobile phase, HPTLC produced an excellent separation of TMP on silica gel F254 plates. The linearity, precision, limit of detection, and limit of quantification of videodensitometric quantification were all confirmed. The LOD was 0.05  $\mu\text{g}$  per spot. Extracts from spiked water samples were obtained through solid-phase extraction. Using HLB cartridges and acetonitrile elution produced the best recovery of these antibiotics. The results showed that the apparent recoveries for trimethoprim was  $108.7 \pm 23.7$  [55].

The analysis of antibiotics in environmental waters is difficult due to the complex matrices under investigation and the low concentrations ( $\text{ng/L}$ ) of target compounds that are typically present. This makes the creation of extremely sensitive analytical methods necessary for the monitoring of these analytes' low  $\text{ng/L}$  concentration levels. Because antibiotic concentrations in the environment are typically low, preconcentration is an essential step before detection. Many methods for locating the most important members of this pharmacological class in diverse environmental samples were investigated due to the pervasive problem of drug contamination and environmental risk assessment [56]. LC analysis is usually performed after off-line SPE, which is the recommended technique for sample pre-concentration.

The aquatic environment contains a wide variety of antibiotics from various classes. As a result, multi-residue analytical techniques are currently favored for the identification and surveillance of various antibiotic classes. To analyze environmental samples, these techniques need to be sensitive, selective, quick, and simple to use.

Reviews on the identification of particular classes of pharmaceuticals have already been published in several academic journals [57,58]. In this review, we aim to compile analytical techniques that have been published for the determination of specific antibiotics in environmental water samples.

### **Conclusion**

Antibiotics are being used in more fields than ever before, which has led to the development of novel sample preparation and determination techniques for measuring and identifying antibiotics. An overview of the most recent methods developed for identifying the different antibiotics found in the environment is given in this work. Following the pre-concentration stage, the extracted metronidazole and trimethoprim are typically identified using different analytical techniques. Many techniques for determining the presence of these antibiotics in environmental samples have been published. The recommended method combines mass spectrometric detection with liquid chromatography due to its increased accuracy, precision, and lower detection limits.

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