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Development of Analytical Technique for Extraction of Commonly used Antibiotics in River Yamuna Based on liquid-liquid extraction

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ABSTRACT

Concentration of pharmaceutical especially antibiotics in different ecosystems of environment is incredibly challenging. To enable quick, sensitive, and targeted determination at trace levels, designing of appropriate analytical methods is becoming important. Different techniques like liquid chromatography-tandem mass spectrometry (LC-MS/MS) and gas chromatography-mass spectrometry (GC-MS) have been used widely. One of the environment friendly technique, Liquid-liquid extraction (LLE) was used for the extraction of some commonly used antibiotics in different samples of river Yumana, because of its cheap and easy extraction procedure. To achieve the goal of extraction, variety of operational parameters were optimised, that are responsible for increasing the chromatographic resolution, sensitivity, and accuracy. LC-MS and LLE methodologies can be used as an essential tool for researchers for their efforts to quantitatively analyse antibiotics and other related emerging contaminants in the selected environmental samples.

Keywords: Antibiotic residues, Analytical techniques, Extraction, Liquid chromatography-tandem mass spectrometry (LC-MS/MS).

INTRODUCTION

Preventing the spread of harmful microbes or to kill them, several natural and artificial pharmaceuticals are used which are typically referred as "Antibiotics"[1]. Drugs especially antibiotics are enormously in use in different sectors of the environment such as fish farming and agricultural industries. Recently the excessive intake of drugs has increased the discharge rate into the sewage networks, where they may find their ways to surface waters[2]. High antibiotic concentrations are found in treated sewage that are currently causing concern around the globe, owing to their extensive usage in immense quantities and potential to cause bacterial resistance[3]. As per the report of central pollution control board the river Yamuna is significantly polluted and 40% of direct discharge

from surface runoff, drains carrying effluents from industries, and blighted areas along the edges of river are the major cause of pollution[4]. According to several researches, human usage is the primary sector that increases the discharge of drugs and their metabolites into the aquatic ecosystem[5]. A further concern is raised by the fact that certain drugs that persist in the aquatic ecosystem, repeatedly appear in soil, sediments, surface water and even biota. Pharmaceuticals are not removed completely by wastewater treatment plants, which allows them to enter aquatic environment[6]. After being excreted from the body, dumped into landfills and water bodies, majority of these chemicals have unpredictable biochemical interactions when combined, and even at very low concentrations remain bioactive, starts accumulating in the food chain with detrimental effects on health of living organisms[7]. As the consumption rate increases they are becoming more and more noticeable[8]. However, quantification and identification of pharmaceutical residues like antibiotics in different environmental matrices is becoming very challenging. Low analyte concentrations and complicated matrix effects provide a great challenge[9]. To conduct an accurate analysis, pretreatment stages are frequently necessary, during which the analyte must be separated from confounding matrix components and then concentrated for specific target drug analysis[10].

Criteria for the selection of antibiotics

The drugs were chosen from the exhaustive literature review based on various prioritisation factors. Approximately 12 pertinent criteria have been selected based on logical and scientific justifications. Priority has been given to the chosen drugs since they are high priority pharmaceuticals and are used more frequently and regularly in the environment. Among many drug prioritizations methods, rate of consumption and/or discharge are some of the basic criteria that have been used for selection of drugs[11,12]. Bioaccumulation potential which acts as a fundamental risk factor for aquatic life, is also included as an important criteria for the selection of these drugs[13]. The cretria considered and the condition to fullfill these cretria are explained in the Table 1.

Table 1: Conditions for fulfillment of the *Cretrias*

S. No	Selection criteria	Condition for the fulfilment of the criterion
1	Intake of drugs in multi-specialty hospitals	Drugs that are consumed very often and in greater amounts.
2	Regulation	Drugs recognised under international law
3	Metabolism	Formation of active metabolites
4	Excretion factor (% of drugs excreted)	An extra risk exists when the excretion factor is more than 10%.
5	Toxicity/eco-toxicity	Support from the literature for the toxicity of drug residues.
6	Presence in different aquatic ecosystems	The reported drug residues in aquatic ecosystems are considered.
7	Physical and chemical properties	The properties that improve the aquatic ecosystem's ability to adsorb drug residues from water and sediment.
8	Bio-concentration factor	Drugs with Bioconcentration factor value of <100- are not. expected to bio-accumulate Drugs with Bioconcentration factor value of >100 but <1,000 - has the potential to bio-accumulate[13]. The drugs with Bioconcentration factor value of >1,000- has the significant bio-accumulation potential
9	Value of octanol water partition coefficient (Log Kow/ Log P)	The below are the listed criteria's for determining the potential of adsorption for drugs Low potential for adsorption when Log Kow <2.5 Moderate potential for adsorption when Log 4.0< Log Kow> 2.5 High potential for adsorption when Log Kow > 4.0
10	Value of water-organic partitioning coefficient in soil (Log Koc)	For sediment effect assessment, a log Koc ≥3 is taken as a trigger value and the sediment compartment contains persistent drugs with such values.
11	Resistant to treatment	Consideration of drugs that are resistant to the conventional treatment technologies
12	Persistent	Persistent drugs in aquatic environment have higher bio-concentration values.

Development of analytical Techniques

Availability of testing methods that are more reliable and sensitive is crucial for regular monitoring and is required to prevent antibiotic residues from entering into the aquatic environment[14]. Moreover, the simultaneous study of numerous groups of compounds with diverse physicochemical characteristics sometimes calls for the careful selection of experimental conditions. To enable quick, sensitive, and targeted determination at trace levels in environmental samples, designing of appropriate analytical methods is becoming essentially important. There are numerous analytical procedures that are expressed in the literature for the detection and estimation of varying concentration of emerging contaminants in surface water, wastewaters, soil, and other biota[15].

Different techniques based on liquid chromatography (LC-MS/MS) and Gas Chromatography (GC-MS) for the evaluation of antibiotics have been reported[16]. However, due to greater selectivity and compatibility with the antibiotics having higher polarity and low volatility, LC-MS/MS has more often used than GC-MS[17,18]. Optimising several operational parameters that could enhance the resolution power of chromatography, its sensitivity, and accuracy for the analysis of selected drugs are crucial to achieve this goal, and to guarantee their correct detection and quantification at different concentrations. The established analytical methodologies will be an essential tool for researchers to use in their efforts to quantitatively analyse antibiotics and other related emerging contaminants in the selected environmental samples[19].

Various types of extraction techniques

The initial process that helps in separation of desired product of interest from the raw ingredients is “Extraction.” There are different traditional and modern processes of extraction. The traditional method of extraction involves the extraction using reflux, percolation, and maceration processes, that frequently employ the use of large quantity of organic solvents and takes long duration to complete the process. Modern method of extraction, such as solid phase extraction and liquid-liquid extraction etc., are the recent and more environmentally friendly techniques, extensively used to extract natural and targeted products[14,20,21]. These techniques have advantages over older ones, including consumption of less organic solvent, quicker extraction duration, and greater selectivity. In present times, numerous sample extractions methods are in use for the extraction of biological samples. Liquid-liquid extraction (LLE), solid-phase extraction (SPE), solid-phase microextraction (SPME), stir-bar sorptive extraction (SBSE),pressurised liquid extraction etc., are examples of the extraction techniques that are most widely used nowadays[22]. To extract desired compound of interest, the solvent must be carefully chosen. Considerations like selectivity, solubility, cost, and safety should be made before the solvent selection. According to the similarity and dissimilarity rule, polar solvents having values near to those of the solute will perform better function. Polar value of solvents close to the polar value of solute are likely to show better results and vice versa[23]. Ethyl and methyl alcohols are the most used extraction solvents. Diffusion and solubility both rise with the rise in temperature. However, when the temperature is very high the loss of solvent lead to the extraction of undesired contaminants and the destruction of thermolabile components[1].

Liquid-liquid extraction

Most established and popular methods for preparing materials for qualitative and quantitative analysis is liquid-liquid extraction or previously known as solvent extraction. Sample components are divided between two immiscible liquid phases. The procedure is carried out in a separatory funnel in its most traditional form by agitating the mixture to disseminate drops of one liquid in the other, reducing agitation to allow for drop crystallization, then separating the bulk liquid phases from one another[24].

This method makes the most extensive use of solvent extraction. Steps involved in the extraction of natural products are as follows: (1) enabling the solvent's penetration into the solid; (2) allowing the solvents to dissolve the solute; (3) enabling the solute to flow out of the solid material; and (4) collecting the solutes that were extracted. In liquid-liquid extraction, the quantity and the type of extraction solvent significantly affects the analyte recoveries[21]. The optimum extraction solvent should be denser than water, less soluble in water, and capable of extracting a substantial amount of analytes[25]. An extraction process is made easier by the substance that enhances the permeability and solubility in

the procedures. The extraction efficiency is greatly influenced by the characteristics of solvents, including raw material size, solvent/solute ratio, temperature, time, etc[26,27].

Solid-Phase Extraction (SPE)

With the use of solid phase extraction technology, multiple compounds and high target analytes can be obtained. This method have shown great affinity for target analyte extraction, preconcentration, and cleanup from complex aqueous matrixes[10]. SPE involves the supply of mobile phase through a cartridge containing a solid stationary phase to extract the drug that has been dissolved from the mobile phase. A suitable solvent is used to condition the cartridge, wetting the stationary phase surface[27]. Once the sample is put into the cartridge (mobile phase), the desired analytes are kept, and the solvent allows the remaining undesired matrix chemicals to pass through. Selected drugs are then rinsed in a solvent that is frequently buffered to the right pH after the cartridge has been thoroughly cleaned to remove contaminants[28]. The solvent is then prepared for instrumental analysis after being eluted and adjusted to a certain volume.

Solid-Phase Microextraction (SPME)

For sample preparation, Solid Phase Microextraction techniques offer straightforward, solvent-free procedures that have been applied to the identification of several antibiotics. Solid phase microextraction (SPME) collects and preconcentrates target analytes using a coated fiber. After that, samples are calibrated. Instrumental analysis is carried out after the thermal desorption of analytes into the analytical device or into an organic solvent[15]. The advantages of solid phase microextraction are low sample volumes, simple and effective processing, and extraction over SPE. Increased detection limits and matrix effects are drawbacks[28]. SPME takes a lot of time, and sample carryover is a possibility. Additionally, in this procedure most of the fibers used are expensive, delicate and have short lifetime[1].

Stir-bar Sorptive Extraction

To reduce waste, current advances in analytical chemistry emphasize the downsizing of these procedures and the quantity of harmful reagents. Due to its ease of use, high extracting capacity, and environmental friendliness, stir bar sorptive extraction, has been widely used for enriching organic contaminants in various types of matrices[29]. Currently due to numerous benefits, such as fast, great sorption capacity, persistent, and great extraction efficiency, stir bar sorptive extraction has generated a lot of interest. The application of SBSE enables the creation of analytical procedures with low limits of detection, less than ng/l concentrations, for a variety of nonpolar species (log Kow>3). This has led to its use in the analysis of numerous materials, like food, water, and other biological samples[30]. For strong polar or hydrophilic solutes extraction by this technique is still a difficult problem. There are only three stir bar extraction coatings that are typically used: polydimethylsiloxane (PDMS), polyethylene glycol (PEG), and polyacrylate (PA). Given the narrow selection of stir bar sorptive extraction coatings that are commercially accessible, much research effort has been put into creating new coatings to increase the technique's flexibility and possible uses[31].

Pressurized liquid extraction

An exhaustive method of extraction usually referred as pressurized liquid extraction (PLE). The method is thought to be relatively simple, easy learning, effective, and offers good number of recoveries within a short time of technique development[32]. After introducing sample, the cell is rotated and combined to inert substance, the sample is set into position on the carousel in such way that it may be transferred into the oven chamber. Inside oven, cell is heated and pressured before being automatically shut under pressure[33]. The oven's temperature and pressure are maintained at a consistent level once the solvent fills the cell and is left there for some time. In a vial the solvent is collected, and the cell is finally purged and flushed with the nitrogen gas. Typically, the extraction process takes 15 to 45 min, but sometimes it takes longer extraction time[32].

High temperatures (often up to 200°C) and pressures typically up to (200 bar) are used by Pressurized liquid extraction (PLE) without exceeding the critical point. Pressurized liquid extraction (PLE) uses modest quantities of organic solvents to extract rapidly. It offers recoveries comparable to those of other methods. The factors that have a substantial impact on the recoveries of solvent are temperature, pressure, static extraction duration, number of rotations, and sample weight[34].

Advantage and limitations of different extraction techniques

Method	Advantage	Limitations	References
Liquid-liquid extraction	Liquid-liquid extraction is the most common techniques for sample preparation, higher selectivity, cost effective, ease of implementation, quick collection, and measurement of the organic phase adaptable with analytical devices such as capillary electrophoresis, liquid chromatography (LC), and gas chromatography (GC).	Increased time duration and the amount of solvent, loss of analytes, contamination of samples, and low sensitivity. Formation of emulsion difficulties in the phase separation, low precision in the analysis of and use of large volumes of toxic organic solvents, time-consuming process, complex matrix samples.	1
Solid-phase extraction	SPE, has emerged as an effective alternative approach due to its ease of use, adaptability, and high sample throughput. It involves the use of cartridge, so the interference is removed, higher selectivity, increased sensitivity, cleaner extracts, lower solvent consumption	Effort involved for the offline SPE, when many samples are taken frequently, poor reproducibility, greater loss during evaporation for polar and nonvolatile compounds, disposable cartridge for handling water samples reduces the consumption of organic solvents, cost of sorbent materials is still higher	33
Solid phase microextraction	Non exhaustive, modern method, easy procedure, low-cost, small use of organic solvent or solvent free extraction procedure, simple and effective processing, versatile, and high throughput sample preparation technique.	For chemicals to be quantitatively kept by the sorbents, the sample volume must be less than the retention capacity of the sorbent., limited volume of extraction drops, limited choice of extraction solvents, in water, partial solubility of organic solvents.	15
Stir-bar Sorptive extraction	First modern solventless extraction technique for organic compounds, fast, great sorption capacity, persistent, and great extraction efficiency, applicable to volatile organic compounds, and semi volatile compounds, more effective for nonpolar species.	Process of multiple polymerizations, which is often required to maximise the adsorption capacity, ineffective for strong polar or hydrophilic solutes, expensive equipment's required, limited and expensive coatings available, less flexibility.	31
Pressurized liquid extraction	Environmentally friendly extraction techniques, increasing automation, extraction time very short and decrease in the number of organic solvents, uncomplicated, exhaustive, easy to learn, low cost, high sensitivity, repeatability, short preconcentration time, applicable for water, soil, sediments without further. cleanup	Extraction efficiency towards the analytes is not as high as might be desired, effect due to temperature and pressure on the efficiency of extraction, expensive equipment's, low selectivity, dilution of analyte.	33

Chemicals, standards, and reagents

In this study, the high purity standards of antibiotic compounds, selected for analysis, were purchased from Sigma-Aldrich chemicals, Bengaluru (India). The purest possible grade was used for each standard that was employed in this study. Except for water and all the solvents used for liquid-liquid extraction (LLE) and UPLC-MS analysis were of UPLC quality. The calibration standards and reagents were prepared using Milli-Q water. Filtration was done using disposable syringes of 3 mL capacity and pore size of 0.45µm sterilised syringe filters as described by[35,36]. To extract the antibiotics, analytical-grade Chloroform was used. The laboratory's distillation equipment provided the distilled water needed for the rotary evaporator[37].

Extraction method for water samples

For analysis of surface water and wastewater samples of river Yumana, 500 mL of water sample is processed by adding 200 mL of chloroform and leaving them in an incubator at room temperature for 30 minutes. The analysis was done according to the procedure carried out by[18]. Separated through separation assembly, the upper extractant phase contains antibiotics and the lower phase is the wastewater. To extract antibiotics completely, the process is repeated twice. The next two repetitions involve the further addition of 50 mL of chloroform to the lower layer containing antibiotics and are placed in an incubator for at least thirty minutes. So, in the end, 300 mL of chloroform was used. In rotary evaporator, at a temperature of 70°C, the sample was dried near dryness with further addition of 2.5 mL of methanol to the solid portion. The sample was then injected via syringe of 3 mL into the UPLC sampling vials using 0.45 µm sterilized syringe filters[26]. Further analysis was done as per the optimised protocol of UPLC-MS/MS.

The Fig.1(a) shows the method of extraction as per the optimised protocol, Fig. 1(b) shows the concentration of one of the analysed drugs Ofloxacin in water samples of river Yumana during pre-monsoon and post-monsoon period along with the chromatogram shown in Fig. 1(c). Qualitative analysis of drugs was done by comparing the molecular mass from the chromatogram. The further estimation and production were carried out by quantitative analysis via estimating the concentration of precursor ion having (m/z=362.3) and (m/z=318.3) respectively.

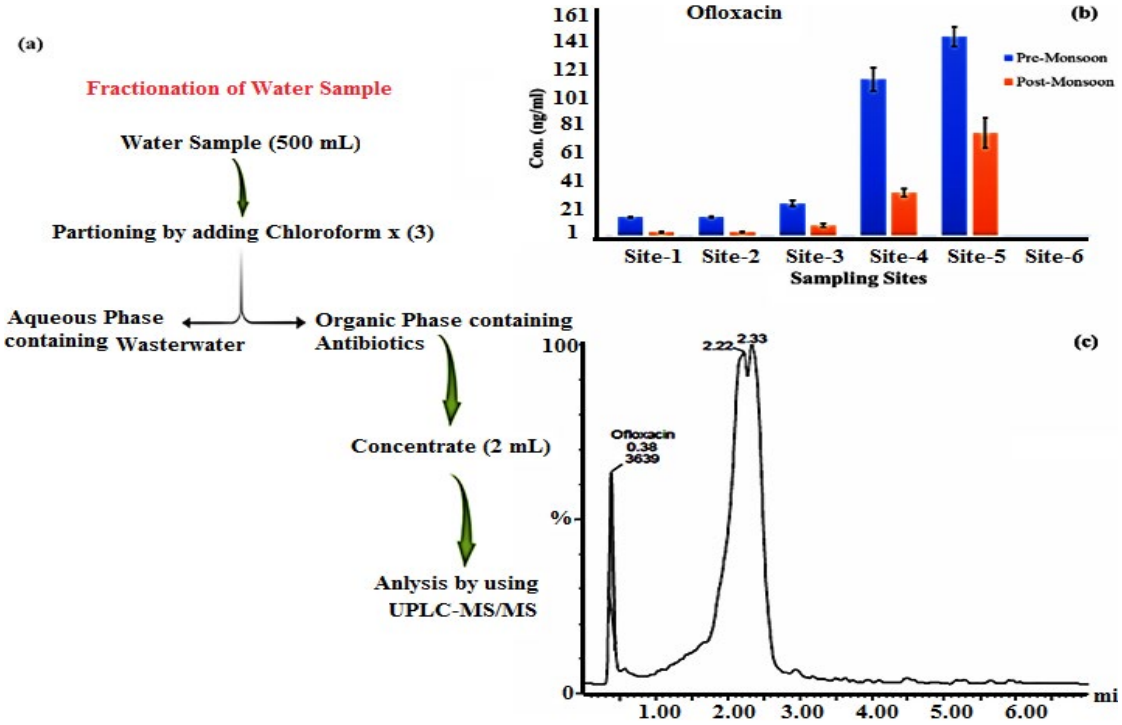

Fig. 1(a). Systematic representation of extraction procedure (b) concentration at different sampling locations (c) chromatogram showing quantification of the ofloxacin antibiotic in surface water

Extraction method for soil samples

For analysis of antibiotics from the soil, sampling was done from the agricultural land irrigated via water from river Yumana. Samples were taken from top layer (5-10) cm by a grab of stainless steel, wrapped in aluminum foil and sealed in the polythene bags before storage. 100 g of soil is processed by adding 200 mL of chloroform and leaving them in an incubator at room temperature for 30 minutes. The analysis was done according to the procedure performed by[18,23]. Sonicated for 2 h by using ultrasonication, concentrated and fractionated in the ratio of 1:5. Acidified with 0.1 NHCL upto pH 5. Portioned by adding chloroform. Wastewater present in the lower phase (aqueous layer) while the antibiotics are present in the top phase extractant. To completely extract antibiotics the process is repeated thrice. The next two repetitions are performed by adding 50 mL of chloroform to the extracted phase (lower phase) that contains antibiotics. Basified with 25% ammonia upto pH-11, again the water sample is separated through separation assembly, by adding chloroform. Now this time the upper phase (aqueous layer) is the wastewater and the lower phase (organic layer) extractant contains antibiotics. The repetition of the process occurs twice to extract antibiotics completely. Further 50 mL of chloroform was added to the next two reparations and was placed in an incubator for thirty minutes, at the end, final volume of chloroform used are 300 mL. In the rotary evaporator the sample was dried to near dryness at temperature 70°C and 2.5 mL methanol (CH_3OH) was added to the remaining residue by using sterilised syringes of 3 mL capacity and 0.45 µm syringe filters, the sample was then injected to UPLC sampling vials[26]. The samples were then analysed via UPLC, LCMS/MS as per the optimised protocol[18].

The Fig. 2(a) shows the extraction method of selected antibiotics, Fig. 2(b) shows the concentration of one of the analysed drug Amoxicillin in soil samples of river Yumana during pre-monsoon and post-monsoon period along with the chromatogram Fig. 2(c). Qualitative analysis of the antibiotic was done by comparing the molecular mass from the chromatogram. The further estimation of antibiotic was calculated via quantitative analysis by estimating the concentration of precursor ion having (m/z=366.2) and product ion (m/z=114) respectively.

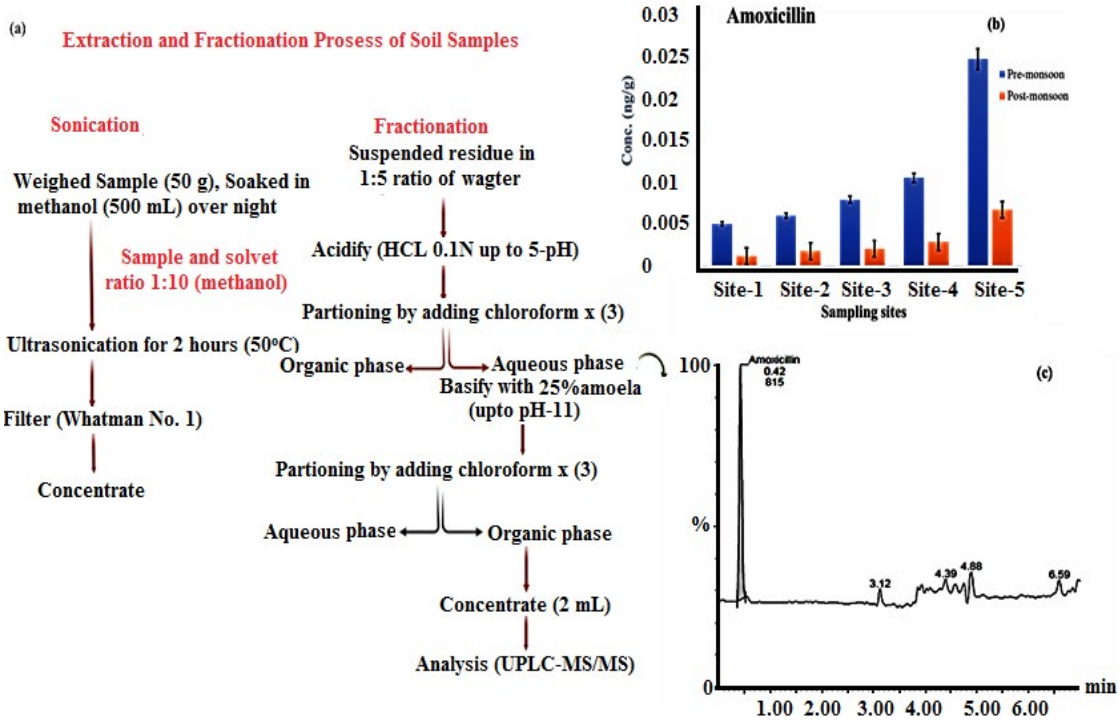

Fig. 2(a). Systematic representation of extraction procedure (b) concentration at different sampling locations (c) chromatogram showing quantification of the Amoxicillin antibiotic in soil samples around river Yumana

Extraction method for vegetable samples

The application of reclaimed water in conjunction with the use of sludge or manure to agricultural areas as fertiliser may explain the

presence of some commonly used antibiotics into the soil[38,39]. The transfer of emerging pollutants (antibiotic) from the reclaimed water to crops via plant uptake is a major public issue about agricultural uses of treated water and biosolids. Human health hazards might arise from these substances being present in food[40]. For analysis of drug residues in freshly grown vegetable, (*Spinacia oleracea*) was handpicked from agricultural field, collected in polybags, and then transported to laboratory where they are washed by tap water to remover mud, sand and impurities, shade dried at room temperature before carried out for analysis. 5 g of sample was processed by adding 50 mL of chloroform and leaving them in a shaking incubator at room temperature for 30 minutes. Sonicated for 2 h by using ultrasonication, concentrated and fractionated in the ratio of 1:5. Acidified with 0.1 N HCL upto pH 5 and was portioned by adding chloroform. Aqueous phase (Lower layer) contains the waste material while the desired materials are present in the top extractant layer[41]. To extract antibiotics completely this process is repeated twice further. Again, the next two repetitions were accompanied by the addition of 50 mL of chloroform to the lower phase containing antibiotics. Basified with 25% ammonia upto pH-11, again separated through separation assembly, by adding chloroform 50 mL. Now, the upper phase (Aqueous layer) is the wastewater and the lower phase (Organic layer) extractant contains antibiotics[18]. The repetition of the process occurs twice to extract antibiotics completely. Further 50 mL of chloroform was added to the next two reparations and was placed in an incubator for thirty minutes each, at the end, final volume of chloroform used are 300 mL. In the rotary evaporator the sample was dried to near dryness at a temperature of 70°C and 2.5 mL of methanol (CH_3OH) was added to the residue portion, and by using sterilised syringes of 3 mL capacity and 0.45 µm syringe filters, the sample was then injected to UPLC sampling vials[26,42]. The samples were then analysed via UPLC, LCMS/MS as per the optimised protocol.

The Fig. 3(a) shows the method of extraction, Fig. 3(b) shows concentration of one of the analysed drugs Erythromycin in vegetable samples irrigated through the water supply of river Yumana during pre-monsoon and post-monsoon period along with the chromatogram Fig. 3(c). Qualitative analysis of the antibiotic was done by comparing the molecular mass of antibiotic from the chromatogram. The further estimation of antibiotic was calculated via quantitative analysis by estimating the concentration of precursor ion having (m/z=734.5) and product ion (m/z=158.1) respectively.

5.4. Sample analysis by using UPLC-MS/MS

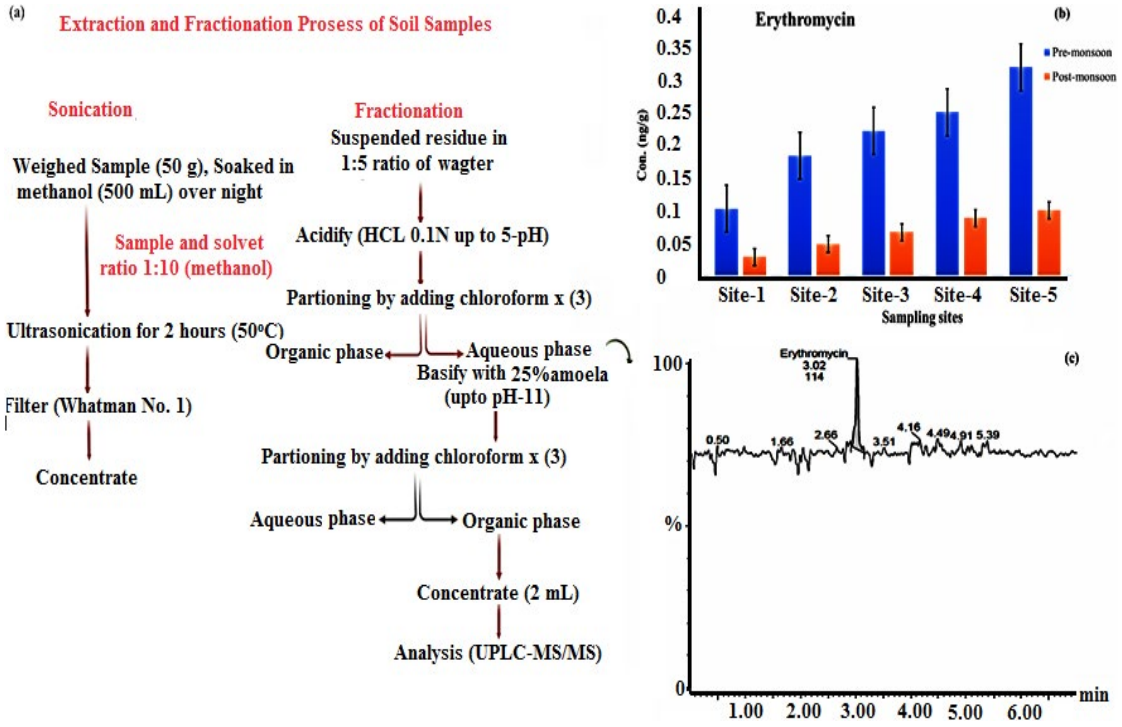

Fig. 3(a). Systematic representation of extraction procedure (b) concentration at different sampling locations (c) chromatogram showing quantification of the Erythromycin antibiotic in vegetable samples irrigated by the surface water of river Yumana

Using a Waters UPLC H-Class separation module, the liquid chromatogram separation was carried out (Milford, MA, USA). Likewise, ACQUITY UPLC BEH C18 (2.1 mm i.d. x 50mm, particle size 1.7 m) was used. LC columns was evaluated using 0.1 percent formic acid in ultrapure water as eluent "A," whereas 0.1% formic acid in ultrapure acetonitrile used as eluent "B". The produced mobile phase was ultrasonically processed through 0.22 m sterilised cellulose nitrate filter paper after being sonicated at room temperature for 10 minutes. To improve the approach, numerous runs were made under various circumstances. These included variable flowrates, temperatures, and mobile phase compositions. The gradient that produced the best separation began with 90 percent of eluent A, was held for 1 min to 90 percent of eluate A. From 1 to 2 min, it was gradually changed from 90 to 50 percent of eluate A, and for another 2 min to 20 percent of eluate A with was held for another one minute, then changed to 90 percent of Eluent A in 0.1 min which was then kept at the same condition at 90 percent eluent A for another 2 minutes. The injection has a 10 µL volume. Waters UPLC H-Class with quaternary pump having Photodiode detector (PAD), ACQUITY UPLC BEH C18 (2.1 mm i.d.x50mm, particle size 1.7 m) was used. LC column and auto sampler for auto injection were used to perform the tandem mass analysis. With the use of these instruments, the procedure was optimised and a universal method for identifying all three chosen antibiotics was created. Seven distinct concentrations, ranging from 5ng/ml to 200 ng/mL, were made for each standard stock solution. Each concentration was passed through the UPLC at least thrice, to evaluate the consistency, peak regions, and the retention time of the system. Plotting of the concentration versus peak area produced calibration curves. Through stock solutions with larger concentrations of pure antibiotic standards, the specificity was achieved. The absence of contaminants was confirmed by the single sharp peak, which also assisted in calculating the retention time. Confirmation the peak region and the retention time was done at least five times. Analyzing actual and spiked (selected antibiotics) water samples helped to further confirm the procedure. The standard deviation of response and slope of the curve were used to assess the limit of detection (LOD) and limit of quantitation (LOQ) using the calibration curve i.e. LOD=1.5 ng/mL.; LOQ=5 ng/mL[20].

Mass spectrophotometer

Using liquid chromatography and the Waters Xevo TQ-S Micro and UPLC H-Class, the medication concentration was analysed (Tandem Quadrupole Mass Spectrometer). A positive-mode electrospray ionisation source (ESI) assists the UPLC in identifying and quantifying the chemical. To optimise the dependent parameters, a direct infusion of a 10 µL/min of standard solution of all the analytes was employed. Selection of ionisation and precursor ion mode was the next step to follow. The product ions and their corresponding capillary voltage was 4.5 kV, 150°C temperature of source, desolvation temperature was 450°C, flow of cone gas was (50 l/h) and flow of desolvation gas was (850 l/h). By using the automated quantitative optimization method, all these factors were determined[43]. Simultaneously, the quantification and confirmation of analytes transitions were done, the precursor ion and the product ions selected for the quantification transition showed the strongest response and highest sensitivity.

For the sample analysis, the following were the ideal run circumstances: A precise flow rate of 0.35 mL/min was used to inject 10 µL of the sample onto the UPLC column. As the column setting was "not controlled," the temperature was 40°C. Peak areas of actual samples were compared with the peaks areas of standard solutions for which the concentration of antibiotics were measured, as described in Eq. 1, and by using Eq 2 the response factor was calculated.[44,45]

$$\text{Analyte amount} = \frac{\text{Peak area of the Sample}}{\text{Response Factor}} \quad (1)$$

$$\text{Sensitivity factor} = \frac{\text{Standard peak area}}{\text{Amount of Response}} \quad (2)$$

CONCLUSION

For the estimation of pharmaceutical like antibiotics in different environmental matrices, various analytical methods have been recently developed. Extraction and analysis of multi classes of compounds simultaneously is a great challenge and is presented because of broad range of polarities, solubilities, partition coefficients and various other characteristic under the acidic and basic conditions. Developing suitable methodology using liquid chromatography (LC) mass spectrometry (MS/MS) in positive ionisation (PI) mode for the determination of

selected therapeutic class of drugs was the purposes of this work. By altering the mobile phase's gradient and composition and employing various LC columns, the goals were to ensure that the compounds would be separated, to maximize the method's sensitivity by modifying variables such collision energy in tandem MS, and to enhance Liquid phase extraction (LPE) recovery. The procedure was then prepared for the analysis of various environmental sample.

Furthermore, the simultaneous investigation of three different classes of pharmaceutical compounds with various physicochemical properties typically necessitates the choice of experimental design carefully. It was essential to achieve this goal by optimising a variety of operational parameters that are responsible for increasing the chromatographic resolution, sensitivity, and accuracy of the selected drugs and to guarantee their correct detection at different concentrations. These analytical techniques will become a crucial method for water corporations to use in their attempts to remove drug residues and other similar emerging pollutants in different ecological samples.

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Conflicting interest

The authors affirm no financial or interpersonal conflict that have any impact on the research presented for publication.

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