FINAL REPORT ON

UGC MAJOR RESEARCH PROJECT

(01.07.2015 to 30.06.2018)

Entitled

"Development of Preconcentration Methods for the Analysis of Drugs in Environment samples"

(F. No.-43-234/2014 SR)

Submitted to

University Grants Commission (UGC)

Bahadur Shah Zafar Marg

New Delhi 110-002

Submitted by

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Annexure IX

UNIVERSITY GRANTS COMMISSION

BAHADUR SHAH ZAFAR MARG
NEW DELHI – 110 002
PROFORMA FOR SUBMISSION OF INFORMATION AT THE TIME OF SENDING THE

FINAL REPORT OF THE WORK DONE ON THE PROJECT

1	TITLE OF THE PROJECT	Development of Preconcentration Methods for the Analysis of Drugs in Environment samples
2	NAME AND ADDRESS OF THE PRINCIPAL INVESTIGATOR	Dr. Jatinder Singh Aulakh **Address:** **Office:** Assistant Professor, Department of Chemistry, Punjabi University, Patiala-147002, Punjab **Residence:** 105, phase I ,Urban Estate Patiala
3	NAME AND ADDRESS OF THE INSTITUTION	Department of Chemistry, Punjabi University, Patiala-147002, Punjab, India
4	UGC APPROVAL LETTER NO. AND DATE	43-234/2014(SR)
5	DATE OF IMPLEMENTATION	01/10/2015
6	TENURE OF THE PROJECT	3 years from 01/07/2015
7	TOTAL GRANT ALLOCATED	10,97,150
8	TOTAL GRANT RECEIVED	10,12,995
9	FINAL EXPENDITURE	10,79,030
10	TITLE OF THE PROJECT	Development of Preconcentration Methods for the Analysis of Drugs in Environment samples
11	OBJECTIVES OF THE PROJECT	The objectives of the project: 1. Development and modification of the preconcentration materials by molecular imprinting technique for the drugs. 2. Development of the solid phase extraction cartridges using molecular imprinting polymers and porous molecular imprinted polymers. 3. Application of molecular imprinting solid phase extraction for preconcentration of different drugs. 4. Application of the prepared methods to different environmental samples.
12	WHETHER OBJECTIVES WERE ACHIEVED (GIVE DETAILS)	Yes A molecular imprinted polymer (MIP) for carbamazepine (CBZ) was synthesized with a non-covalent imprinting approach (Bulk imprinted polymerization) so as to facilitate the selective extraction of CBZ from aqueous and bio-samples. It has been also demonstrated that the polymer is able to bind efficiently CBZ and its structural analogue oxcarbazepine present in urine (with a recovery of 90 and 78% respectively) as well as blood samples (with a recovery of 87 and 72% respectively). Porous molecular imprinted polymer (PMIP) was synthesized so as to recognize CBZ, nortriptyline, amitriptyline, diclofenac efficiently from various environmental sample matrices such

		as drinking water, river water, waste water treatment plants, hospital waste water and pharmaceutical samples.
13	ACHIEVEMENTS FROM THE PROJECT	Refer appendix-A
14	SUMMARY OF THE FINDINGS (IN 500 WORDS)	Refer appendix-B
15	CONTRIBUTION TO THE SOCIETY (GIVE DETAILS)	Pharmaceuticals has been considered as one of the most important new class of environmental pollutants. Their occurrence has been reported in natural waters, wastewater, sediments, and sludge. The preconcentration materials developed under the major research project have direct applications for the determination of various drugs present even at trace level concentrations, as our results suggested that the molecular imprinted polymer and porous molecular imprinted polymer provided an effective solution to determine low concentrations of various drug groups in aqueous, bio-samples and pharmaceutical samples etc.
16	WHETHER ANY PH.D. ENROLLED/PRODUCED OUT OF THE PROJECT	Mr. Irshad Mohiuddin, Project Fellow in UGC-MRP is also registered for Ph.D.
17	NO. OF PUBLICATIONS OUT OF THE PROJECT (PLEASE ATTACH)	02 Papers

Appendix-A

Major achievements of the Project:

a) We have developed a fast procedure for the ultra-trace detection of carbamazepine in aqueous environmental samples. A polymer imprinted for carbamazepine has been synthesized via a non-covalent molecular imprinting approach. The molecular imprinting polymer (MIP) has then been applied in a molecular imprinted solid phase extraction (MISPE) protocol, which enables the selective extraction of CBZ from water sample, even when CBZ is present at low concentrations. CBZ recoveries of 97.5% were obtained when water sample was spiked with CBZ (at a concentration level of 10 µg/L) was percolated through the polymer. It has been also demonstrated that the polymer is able to bind efficiently CBZ and its structural analogue oxcarbazepine (OXC) present in urine as well as in blood samples. Therefore, our results suggested that the MIP provided an effective solution to determine low concentrations of CBZ and OXC in aqueous as well as in bio-samples.

b) A porous molecular imprinted polymer (PMIP) was synthesized so as to recognize CBZ efficiently from various sample matrices. In this process, polystyrene spheres were coated with porous silica shells and at a later stage were removed with the help of tetrahydrofuran (THF), so as to add porosity for the polymer which finally contributes towards the high density of recognition sites and efficient binding property for the analytes on the surface. The PMIP-CBZ showed high selectivity, repeatability and good recoveries in determining the CBZ in drinking water (96.5-99.4%), river water (93.2-97.4%), hospital waste water (87.2-91.3%) and pharmaceutical samples (87.5-89.2%). These analysis were done in presence of the other competitor drug analytes.

c) A porous imprinted polymer (PMIP) for tricyclic antidepressants-Nortriptyline (NOR) and Amitriptyline (AMI) was synthesized. The limit of detection and quantification were 0.140 and 0.462 ng/mL respectively. Good recoveries were obtained for drinking water (92-96 %) and hospital waste water (90-94%).

d) A porous molecular imprinted polymer for diclofenac (DCF), which is an important nonsteroidal antiinflamatory drug (NSAID) and widely used to reduce inflammation and as an analgesic in conditions such as in arthritis or acute injury. Limit of detection and quantification

were 0.035 and 0.115 ng/mL respectively. Good recoveries were obtained for spiked drinking water (97-98.3%), river water (92-93.9%) and hospital waste water (91.3-92.1 %).

Appendix-B

A drug is a chemical substance used in therapeutic, treatment and prevention of disease and used to enhance physical or mental well-being. Nowadays, humans and veterinary drugs are used on global scale and in large amounts to combat various diseases. Drugs and their metabolites are continually being released in the environment mainly as a result of manufacturing processes, disposal of unused or expired products and excreta. Because of their physical and chemical properties, many of these substances or their bioactive metabolites end up in soils and sediments, where they can accumulate and induce adverse effects in terrestrial or aquatic organisms. Among these effects, bacterial resistance is increasingly observed and is caused by the growing practice of adding manure and sewage sludge to agricultural fields, which is of particular concern.

Drugs are continually being released in the environment mainly as a result of manufacturing processes, disposal of unused or expired products, and excreta. The widespread administration of these drugs in medicine represents a potential risk, because their residues may persist in edible animal tissues and may result in the development of drug resistant bacterial strains or allergies. Literature on the environmental analysis and occurrence of drugs has addressed a very small percentage of these compounds, so very little information is available about the fate and the potential effects of drugs in the environment. Analytical methods for the detection of pharmaceutical drug residues are existing, although they suffer from the disadvantages of i) limited sensitivity to detect and identify pharmaceutical residues at the environmentally relevant concentration levels, ii) the need of extensive sample preparation to remove interfering matrix constituents, and iii) the use of environmentally non benign solvents in sample preparation.

With the increasing awareness about the health and the environment, there is a dire need to work out newer methods for the trace analysis of drugs in biological and environmental samples. These analysis acts as a guide for the synthesis of safer drugs. The importance of convenient and accurate analytical methods for the analysis of trace levels of drugs in various matrixes is growing tremendously. There has been an increase in interest as to understand their bioaccumulation, mobility and persistence of these drugs in the body and in environment.

Increasing dependence of human on drugs to combat various diseases have resulted in there large scale production. Disposal of large amount of the expired drugs have serious concerns to environment. Development of new preconcentration methods and highly sensitive and selective

detection of drugs is of central importance to drug safety as well as to forensic, biological, pharmaceuticals, and evolutionary studies.

Pharmaceuticals has been considered as one of the most important new class of environmental pollutants. Their occurrence has been reported in natural waters, wastewater, sediments, and sludge. New studies and research reveal their occurrence in samples investigated worldwide. The accurate identification and quantification of pharmaceuticals, particularly in environmental samples can be an analytical challenge, due to the complexity of the matrix and their low levels of occurrence in nature. Several years ago, appropriate analytical techniques did not exist and it has been became really a challenging task for researchers to find out better techniques for the quantification of pharmaceuticals. But nowadays, gas and liquid chromatography (GC and LC) in combination with modern extraction, derivatization, and clean-up methods provide the suitable methods to quantify many pharmaceutical compounds and metabolites down to ng L^{-1} levels. In a constant effort to optimize various analytical techniques, several advances and techniques have recently been developed in equipment and in sample preparation, derivatization, and clean-up procedures. To confront such analytical problems in both GC and LC analytical procedures, a clean-up step is considered mandatory and added before analysis of the final extract. The sample-preparation procedure is the most vital step of the analysis of organic compounds in environmental matrices and samples. The first step in sample preparation is filtration of an appropriate volume of wastewater in order to avoid extraction inefficiencies because of the presence of suspended solids. Thereafter, extraction of pharmaceuticals from the sample into a small volume of solvent is the next step.

Selectivity is probably one of the most important goal in analytical chemistry. It becomes imperative in the case of trace analysis of target species in real matrices, which are often quite complex, as environmental, biological and food samples. Ideally, a working analytical method should be accurate, reproducible and highly selective (or specific) toward the target analytes. In particular, selectivity should be pursued both in the instrumental detection technique and in the sample preparation step. Since the latter is strictly connected with potential matrix effects occurring in the final quantification, and thus with the overall method sensitivity, it appears that selectivity is particularly important in the sample pre-treatment.

Depending upon the sample matrices, there are various techniques for extraction/enrichment/cleanup, the most common being solid-phase extraction (SPE) and solid-

phase microextraction (SPME). Other extraction techniques that have been applied include liquid-phase microextraction (LPME), stir-bar sorptive extraction (SBSE), matrix solid-phase dispersion (MSPD), lyophilization etc. but the selectivity factor is lacking in these techniques and they require extensive optimization of the procedures to minimize adsorption and co-elution of matrix interfering compounds. The development and application of selective sorbent phases are of need of the hour. Solid-phase sorbents (SPSs) have aroused increasing interest in research on sample preparation, as they have key roles in obtaining high clean-up and enrichment efficiency in the analysis of trace targets present in complex matrices. The development of SPSs generally aims for: high selectivity, good adsorptive capacity, enhanced thermal, chemical or mechanical stability and improved lifetime of devices employing them as adsorbent media. There has been much research recently assessing SPSs. One of the SPSs proven useful for pre-concentration and clean-up of pharmaceuticals in aqueous samples are molecular imprinted polymers (MIPs).

In recent years, MIPs proved to be useful materials in several areas of analytical techniques. Molecular imprinting is a process where functional monomer and cross-linker are co-polymerized in the presence of the target analyte which acts as a molecular template. Firstly, functional monomers forms a complex with the imprint molecule, and complying polymerization, their functional groups are postured by the highly cross-linked polymeric structure. Later removal of the imprint molecule leaves cavities with size, shape and chemical functionality complementary to those of the template (Fig. 1).

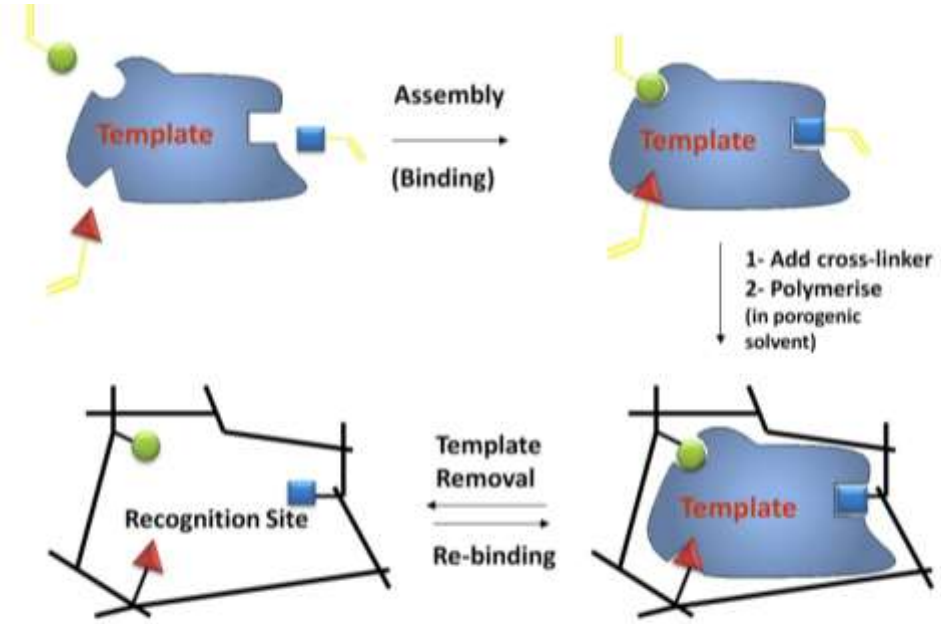

Fig. 1 Schematic representation of the Molecular Imprinting Procedures.

The monomers are chosen by considering their ability to interact with the functional groups of the template molecule. The main advantages of MIPs are their high affinity and selectivity for the target molecule (template). MIPs have higher physical strength, robustness, resistance to elevated pressure and temperature and inertness against various chemicals (organic solvents, acids, bases, and metal ions) compared to biological media such as proteins and nucleic acids. Furthermore, their production costs are low and their lifetimes can be as long as several years at room temperature. The inherently high selectivity associated with MIPs has made them ideal sorbents to be used in SPE, SPME, MSPD and SBSE.

With the increasing awareness about the health and the environment, there is a dire need to work out newer methods for the trace analysis of drugs in biological and environmental samples. The present work therefore, devoted to the development of new methods for the trace analysis of drugs with the help of synthesis of new preconcentration materials by molecular imprinting technique. Work done so far is summarised below:

1. A molecular imprinted polymer (MIP) for carbamazepine (CBZ) was synthesized with a non-covalent imprinting approach (Bulk imprinted polymerization) so as to facilitate the selective extraction of CBZ from aqueous and bio-samples. Synthesised materials were characterized by FTIR and SEM studies. FTIR studies gives a clear indication that MIP for CBZ has been formed,

additionally the SEM images shows the morphology of the imprinted and nonimprinted polymers and these suggests that nonimprinted polymers (NIPs) and MIPs were spherical shape particles (Fig. 2) The imprinted polymer was evaluated and applied as a sorbent for the solid phase extraction (SPE) coupled with HPLC-UV to detect CBZ and oxcarbazepine (OXC)-which is the main metabolite of CBZ. The optimal conditions for molecular imprinted solid phase extraction (MISPE) consist of conditioning of SPE cartridge using triply distilled water followed by methanol, loading of the sample under aqueous conditions and elution by methanol and acetic acid (in the ratio of 9:1). The MIP-CBZ has been applied in MISPE protocol, which enables the selective extraction of CBZ which is even present at trace concentrations. CBZ recoveries of 97.5% were obtained when water sample was spiked with CBZ at a concentration level of 10 µg/L was percolated through the imprinted sorbent in the SPE cartridge (Fig. 3). It has been also demonstrated that the polymer is able to bind efficiently CBZ and its structural analogue OXC present in urine (90 and 78% respectively) as well as blood samples (87 and 72% respectively). Therefore, our results suggested that the MIP provided an effective solution to determine low concentrations of CBZ and OXC in aqueous as well as in bio-samples.

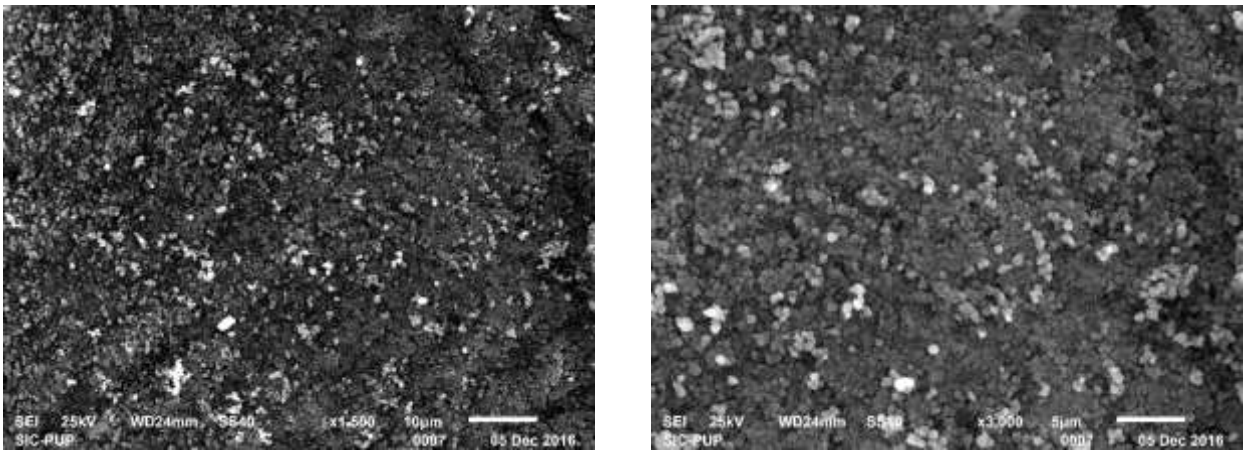

Fig. 2 SEM images shows spherical morphology of the molecularly imprinted polymer.

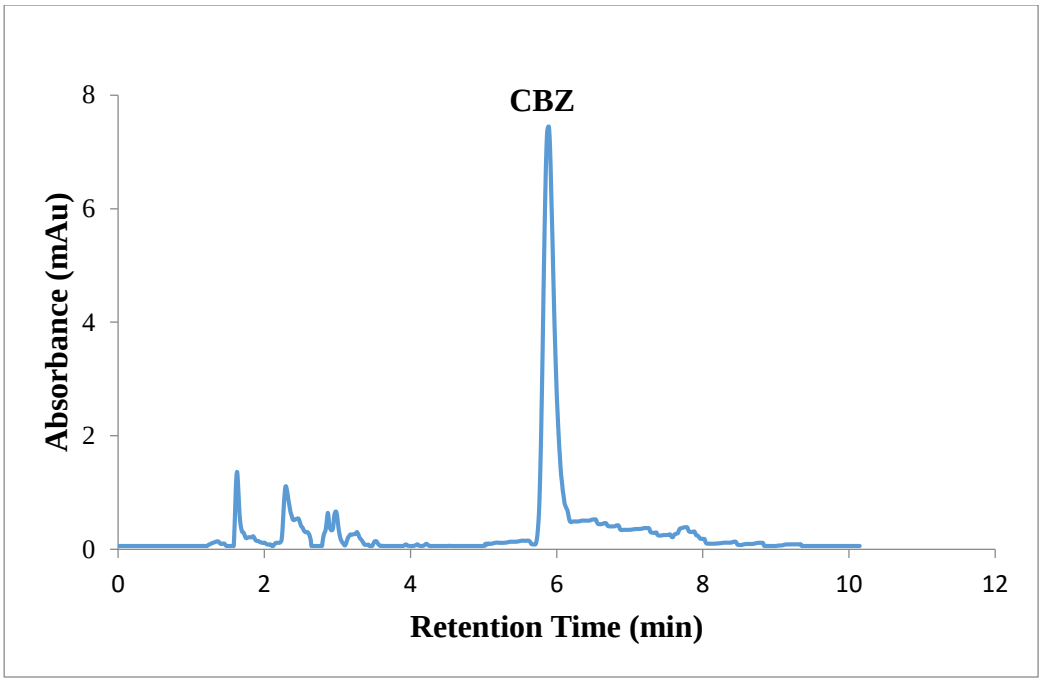

Fig. 3 HPLC Chromatogram for CBZ after preconcentration using MISPE at 5.9 min.

2. A porous molecular imprinted polymer (PMIP) was synthesized so as to recognize CBZ efficiently from various sample matrices. The porous polymer was prepared by a polymerization technique, which so far our knowledge is concerned has not been reported for antiepileptic drug-CBZ. In this process, polystyrene spheres were coated with porous silica shells and at a later stage were removed with the help of tetrahydrofuran (THF), so as to add porosity for the polymer which finally contributes towards the high density of recognition sites and efficient binding property for the analytes on the surface. Synthesized materials were characterized by SEM and FTIR. SEM study shows the polystyrene having a spherical morphology and PMIP for CBZ shows a porous type morphology (Fig. 4). By comparing porous silica and PMIP with the help of FTIR, a clear indication of successful incorporation of imprinted polymer on the surface of porous silica has been demonstrated. The affinity of PMIP-CBZ was confirmed by SPE coupled with HPLC-UV. The major parameters affecting the extraction process, such as, amount of adsorbent to be used in SPE cartridge, eluent type, eluent volume, leakage of the template, flow rate during SPE process, regeneration cycles, cross reactivity were optimized. The calibration curves exhibited good linearity for target analyte with a correlation coefficient value (R^2=0.998). Limit of detection and quantification were 0.082 and 0.270 ng/mL respectively. The PMIP-CBZ showed high selectivity,

repeatability and good recoveries in determining the CBZ in drinking water (96.5-99.4%), river water (93.2-97.4%), hospital waste water (87.2-91.3%) and pharmaceutical samples (87.5-89.2%).

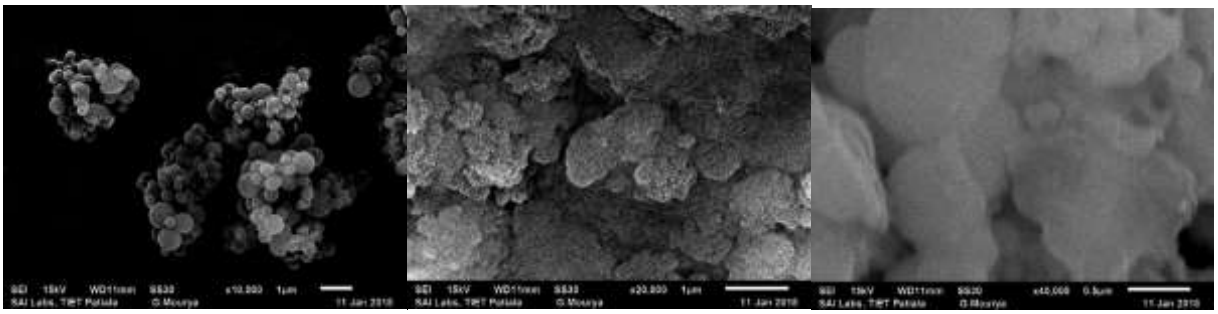

Fig. 4 SEM images of (a) Polystyrene (b) Porous Silica (c) PMIP

3. A porous imprinted polymer (PMIP) for tricyclic antidepressants-Nortriptyline (NOR) and Amitriptyline (AMI) was synthesized. The extraction of these two drugs from aqueous samples was done by SPE using this porous sorbent prepared by a polymerization technique using dispersed polystyrene spheres coated with porous silica layer. SEM study shows the spherical shape of polystyrene and porous (hollowporous) morphology for PMIP. During the evaluation of PMIP-NOR all the experimental parameters were optimized. The calibration curve for the target analyte with a calibration range of 1-250 ng/mL shows a good correlation coefficient value (R^2=0.9983). The limit of detection and quantification were 0.140 and 0.462 ng/mL. The applications of the PMIP-NOR for real samples were analyzed in order to evaluate its performance. Because of the lack of positive results in real samples, so far the rest of aqueous samples were spiked with target analyte at a concentration of 1 ng/mL and was passed via PMISPE cartridge mounted on the SPE manifold and detected by HPLC-UV. Good recoveries were obtained for drinking water (92-96 %) and hospital waste water (90-94%).

4. A porous molecular imprinted polymer for diclofenac (DCF), which is an important nonsteroidal antiinflamatory drug (NSAID) and widely used to reduce inflammation and as an analgesic in conditions such as in arthritis or acute injury. The affinity of PMIP for DCF was confirmed by SPE coupled with HPLC-PDA. The major parameters affecting the extraction process for DCF in aqueous samples were optimized. The optimized parameters include amount of adsorbent, elution solvent, elution volume, flow rate during SPE procedure, effect of salt addition, reusability of the adsorbent, carryover and matrix effects. The optimized SPE and HPLC-PDA conditions were used

to prepare calibration curves for target analytes spiked at 1, 5, 10, 50, 100 and 200 ng/mL concentrations. Good linearity range was obtained for the target analyte with correlation coefficient of R^2>0.99. Limit of detection and quantification were 0.035 and 0.115 ng/mL respectively. The synthesized PMIP-DCF was evaluated for its performance by applying it for the analysis of real samples, and good recoveries were obtained for spiked drinking water (97-98.3%), river water (92-93.9%) and hospital waste water (91.3-92.1 %).

Conclusion

Pharmaceuticals has been considered as one of the most important new class of environmental pollutants. Their occurrence has been reported in natural waters, wastewater, sediments, and sludge. New studies and research reveal their occurrence in samples investigated worldwide. The accurate identification and quantification of pharmaceuticals, particularly in environmental samples can be an analytical challenge, due to the complexity of the matrix and their low levels of occurrence in nature. The sample-preparation procedure is the most vital step of the analysis of organic compounds in environmental matrices and samples. No doubt there are a lot of imprinting studies done for the different drug classes including antiepileptic, antidepressants and non-steroidal antiinflamatory drugs, but there still persists some challenges to this technique such as heterogeneous binding sites, low binding capacity, template leakage, low recoveries, detection levels etc. To overcome such problems porous molecular imprinted polymers were prepared with the help of polystyrene spheres which were synthesized by a facile emulsifier-free emulsion polymerization approach and coating with porous silica was done. There is no work reported so far in the literature as per our knowledge regarding the porous molecular imprinting polymers for these drugs synthesized by the method as worked out in the project. The LODs, LOQs, adsorption capacity and recoveries are quite good as compared to the methods reported earlier. So main aim of the present work was to improve upon the existing imprinting materials to be suited as best adsorbing materials for the sample preparation methods for the detection of different drugs from various environmental sample matrices at trace levels. Due to the outstanding properties of PMIP, we expect it as one of the most promising adsorbent in various preconcentration applications, especially in the analysis of drugs in various environmental samples.