Research Article

Qualitative Analysis of Antibiotic Residues in Hospital Effluents from South India

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ABSTRACT

Large quantities of antibiotics are been used to humans and hospitals are the primary sites of administering antibiotics. Due to continuous release of these antibiotics from hospital effluents into the environment, the aim of this study was to determine the presence of antibiotic residues in hospital effluents from south Indian by High performance liquid chromatography (HPLC) method. The main aim is to determine the presence of antibiotics in the hospital effluent qualitatively by HPLC. A total of ten samples were collected from hospitals of government teaching, government super speciality, private teaching, private super speciality and local clinics for identifying the presence of antibiotic residues and the samples were named as RPA1 to RPA10 respectively. The qualitative analysis of hospital effluent samples for presence of antibiotic residues was carried by high performance liquid chromatography (HPLC) grade methanol and water technique (Hydrophilic chamber technique) for all the ten samples. The results showed that Ciprofloxacin, Enrofloxacin, Oxytetracycline, Trimethoprim and Ampicillin were present in all most all the samples except sample RPA 9. The results indicated that 90% of the samples contained residues of at least one of the investigated antimicrobials. It was concluded that various group of antibiotics were present in the samples collected from hospital effluents. The presence of antibiotics in environment may lead to potential emergence of resistant bacteria that should be watched carefully.

Keywords: Hospital effluents, Antibiotics, HPLC, Qualitative analysis, Antibiotic resistance.

INTRODUCTION

The pharmaceutical and personal care products (PPCPs) are a wide range of organic compounds used for personal health or cosmetic reason including therapeutic drugs.

These substances have diverse physicochemical properties such as partition coefficient octanol-water (K_{ow}), distribution coefficient-biosolids-water (K_p) and solubility that describe the environmental dynamic and in most cases, non-specific biological activity leads to bioaccumulation and enhancement of the emergence of drug resistant microbial species[1,2].

Emerging contaminants have become a serious cause of environmental pollution in the world. Among these, active ingredients of various groups of PPCPs with some metabolites and parent compounds being found in different water bodies on Earth.

Ecotoxicity testing *in vitro* and *in vivo* have demonstrated the toxic effect of these molecules on the food chain[1].

Improper disposal of medicines in hospitals and houses may end up in our water supplies that lead to detection of the traces of pharmaceuticals in the drinking water supplies of various countries[3].

There is a growing evidence that throwing pharmaceuticals (prescription drugs and non-prescription/over-the-counter drugs) and other personal care products in the garbage or flushing them down the toilet or the sink are contributing to this issue, and consequently may have a harmful effect on the environment directly and indirectly on human health. In the past few decades, there has been a dramatic increase in the number of new human and veterinary drugs.

The presence of pharmaceuticals in the environment gaining importance and is a national and international issue that needs to be focused. Although the concentration levels of pharmaceuticals in the environment may be very low, they may be enough to have adverse effects on the environment and on humans. The long term exposure to the trace amounts and mixture of pharmaceuticals may have effect on vulnerable population including pregnant women, new-borns and children[4,5].

Besides the critical role of antibiotics in human health, these antibiotics are potent environmental contaminants so that there has been increasing concern with the presence of different types of antibiotics in the environment[6]. Improper disposal of antibiotics into the environment leads to the emergence of antibiotic resistance bacterial strains. Usually unused therapeutic drugs are sometimes disposed of freely into the sewage system. If the drugs are not degraded or eliminated during sewage treatment, they will reach surface water, ground water and potentially drinking water. There are different pathways for releasing of antibiotics to the environment, unmetabolized antibiotic substances are often passed into the aquatic environment in waste water, misuse of antibiotics for animals, over use in plant fertilization and also improper disposal by hospitals and from households[7]. Our waste water treatment plants are not designed to completely remove antibiotics, and consequently pharmaceuticals released into natural waters. Moreover, antibiotics can pass through all natural filtrations and reach ultimately to drinking water due to their high water solubility and often poor degradability[8].

One of the hazards of presence of antibiotics in effluent from sewage treatment plants may cause increased resistance among natural bacterial populations[9]. Many antibiotic-resistant isolates of microorganisms can be found in the environment and although hit remains controversial, the significant increase in the number of bacterial strains resistant to multiple antibiotics has often been attributed. However, there is concern that long-term, low dose concentrations of antibiotics, such as those present in wastewater and surface water could also result in the development of antibiotic-resistant organisms.

Studies on presence of antibiotic residues in hospital effluent and in other environmental slots have been conducted across the world[10-12]. But in India very few studies estimated antibiotic residues in hospital effluents and other environment samples[13,14]. Furthermore, concurrent studies on antibiotic prescription quantity in a hospital, antibiotic residue levels in its wastewater and resistant bacteria in the effluent of the same hospital are few[15,16].

According to the literature, the extraction of antibiotics from different samples are performed using solid phase extraction technique[17-20] followed by their identification and quantification using different techniques including capillary electrophoresis[17,21], thin layer chromatography[17,20,22] and High performance liquid chromatography[18,21,23,24].

With recent advances in analytical techniques detailed investigation of presence of pharmaceutical compounds in various samples has become possible, high performance liquid chromatography is one among the advanced techniques for determining the pharmaceutical compounds qualitatively and quantitatively[25]. Evaluation of the presence of antibiotics in the above samples will lead to a better understanding of the potential for their release into the environment and their influence towards development of antimicrobial resistance. In the present study, we identified and determined the presence of antibiotics from various hospital effluents using liquid chromatography technique.

MATERIALS AND METHODS

A total of 10 hospital effluent samples were collected from different hospitals of varied locations. Hospital samples were collected from untreated waste water outlets, drainage water, waste outlets from different departments and operation theatres of selected hospitals before water enters the sewer system. The samples were collected into 200ml amber glass bottles with screw cap that were rinsed priorly with methanol and water. Samples were purified through filtration with 0.45µ membrane filters this was necessary to remove solid particles present in them as these particles can alter the pre concentration process on solid phase extraction (SPE) cartridges prior to high performance liquid chromatography analysis. The clean and clear filtrates were collected into clean container and subjected to solid phase extraction.

Prior to HPLC determination, the samples were subjected to analyze turbidity value, turbidity nature and pH of the samples. Turbidity is referring to determining the cloudiness of a solution and it is a qualitative characteristic which is imparted by solid particles obstructing the transmittance of light through a water sample. Turbidity often indicates the presence of dispersed and suspended solids like clay, organic matter, silt, algae and other microorganisms. According to the standard classification of the turbidity nature, the samples were classified into three types including less turbid, moderate turbid and highly turbid[26]. The pH was determined by using pH meter.

The samples were transferred to the lab and stored in the dark at 4°C and extracted within 48 hours. The cartridges were first rinsed with methanol followed by sterile water. The required compounds were eluted from the cartridges and the samples were analyzed by high performance liquid chromatography (HPLC) equipped with UV-visible and photodiode array detector. The analytical column RP-18e 250mm×4.6mm, 5µm manufactured by Merck was used at room temperature for analysis. Approximately 5ml methanol-water (50:50), pH 3.0 used as conditioning solvent, 5ml water (pH 3.0) as washing solvent and 5 ml triethylamine 5% in methanol was used as elution solvent[16]. A total of five antibiotics were selected for chemical analysis based on antibiotic prescription pattern of the ten hospitals and also based on known and suspected environmental impact of various antibiotics.

The limit of detection (LOD) of an antibiotic by the analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value. Several approaches for determining the detection limit are possible. This study highlighted based on visual evaluation and signal to noise approach. The detection limit by visual evaluation is determined by the analysis of samples with known concentrations of analyte and by establishing the minimum level at which the analyte can be reliably detected. The detection limit by signal to noise approach is mainly by determining the signal-to-noise ratio performance by comparing measured signals from samples with known low concentrations of analyte with those of blank samples and by establishing the minimum concentration at which the analyte can be reliably detected. A signal-to-noise ratio between 3 or 2:1 is generally considered acceptable for estimating the detection limit. Based on the Standard Deviation of the response and the slope, the detection limit (DL) may be expressed as 3X standard deviation of low concern/ slope of the calibration line.

RESULTS AND DISCUSSION

All the 10 samples were processed for qualitative evaluation of antibiotic residues in the hospital effluents by high performance liquid chromatography technique. Five antibiotics (Ampicillin, Ciprofloxacin, Enrofloxacin, Trimethoprim and Oxytetracycline) were chosen as marker to evaluate its presence in the samples from different hospitals effluents. The ten samples included in this study were coded as RPA1 to RPA10 respectively. Among the ten samples RPA1, RPA5, RPA6, RPA9 were highly turbid, samples RPA4, RPA8, RPA10 were moderately turbid and samples RPA2, RPA3, RPA7 were less turbid in nature. The nature and pH of the samples were depicted in Table 1, which represents that four samples were acidic in nature and six samples were neutral to alkaline in nature.

The antibiotic residues were detected by HPLC method in all the ten samples included where one sample was identified to have the antibiotics in trace not supported with Method Detection Quantification limits (MDQL) value and the detailed description of the results is tabulated in Table 2. Ciprofloxacin, Enrofloxacin, Oxytetracycline, Trimethoprim and Ampicillin were predominantly identified in 4, 2, 1, 6 and 2 samples respectively. In this analysis molecular weight of the antibiotics were determined by g/mol, the ESI value is detected in all samples, time segment were determined in the range of 1.49 to 13.33 minutes, collision energy as ev, fragmentation amplitude and R^2 also well determined.

The detected antibiotics belong to Fluroquinolones, sulphonamides, beta lactam antibiotics and tetracyclines. As a single and mixed predominant antibiotic, trimethoprim was found among two samples and four samples respectively. The chemical structure and its pictorial descriptions were depicted in Figure 1.

[**A**-Oxytetracycline; **B**-Ampicillin; **C**-Ciprofloxacin; **D**-Enrofloxacin; **E**-Trimethoprim]

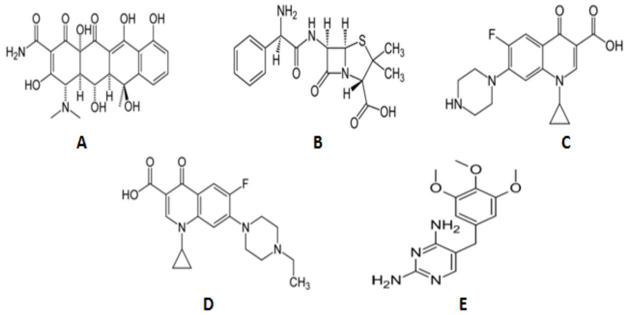

Figure 1: Chemical structure of the antibiotic detected by HPLC

Table 1: Nature and pH value of the Samples

Sample	OD value as TU	Nature	pH
RPA 1	2.7	Highly turbid	6.2
RPA2	0.7	Less turbid	7.4
RPA3	1.4	Moderate turbid	7.3
RPA4	1.3	Moderate turbid	7.2
RPA5	2.9	Highly turbid	6.9
RPA6	3.0	Highly turbid	7.0
RPA7	1.3	Moderate turbid	8.1
RPA8	1.5	Moderate turbid	7.3
RPA9	2.7	Highly turbid	5.9
RPA10	1.1	Moderate turbid	6.5

[The Optical density (OD) value of highly turbid is above 1.5 and above turbidity unit (TU); moderate is between 1 to 1.5 TU and less turbid is below 1 TU]

Table 2: Qualitative detection (LOD) of antibiotic residues by HPLC method

Sample	LOD	Control used	Compound identified	Formula	Mol. Wt (g/mol)	Antibiotic class
RPA 01	Detected	AMP, CIP, ENR, OTC and TMP	CIP, ENR, other trace	C19H22FN3O3, C17H18FN3O3	331.3 359.4	FQ FQ
RPA 02	Detected		TMP, other trace	C14H18N4O3	290.3	DFR
RPA 03	Detected		TMP, other trace	C14H18N4O3	290.3	DFR
RPA 04	Detected		AMP, CIP, other trace	C16H19N3O4S, C19H22FN3O3	349.4 331.3	β lactum FQ
RPA 05	Detected		AMP, TMP, other trace	C16H19N3O4S, C14H18N4O3	349.4 290.3	β lactum DFR
RPA 06	Detected		TMP and other trace	C14H18N4O3	290.3	DFR
RPA 07	Detected		CIP, TMP, other trace	C19H22FN3O3, C14H18N4O3	331.3 290.3	FQ DFR
RPA 08	Detected		OTC, TMP, other trace	C22H24N2O9, C14H18N4O3	460.5 290.3	TC DFR
RPA 09	Detected		Trace	-	-	-
RPA 10	Detected		CIP, ENR, other trace	C19H22FN3O3, C17H18FN3O3	331.3 359.4	FQ FQ

[LOD – Limit of detection; FQ – Fluroquinoloes; DFR – Dihydrofolate reductase inhibitors; TC – Tetracyclines; AMP-Ampicillin; CIP-Ciprofloxacin; ENR-Enrofloxacin; OTC-Oxytetracycline; TMP-Trimethoprim]

In this study, the occurrence of five antibiotics were investigated qualitatively in hospital effluents from south India, very few studies across the India has investigated the residues of antibiotics in hospital effluents, waste water treatment plants, drinking water supplies. From this study, it is clear that antibiotic residues were present in the hospital effluents.

The presence of antibiotics in environments especially of aquatic environment is a matter of concern because of possible development of resistant strains of bacteria.

Study reports showed about prevalence of ampicillin and ciprofloxacin resistant bacteria in river waters, water treatment plants and drinking waters[9,27,28].

Several studies have documented the presence of antibiotic-resistant bacteria in the aquatic environment in general and hospital effluent in particular. It is not yet clearly established that the mere presence of antibiotic residues in the aquatic environment by itself influences the development of resistance, it has been suggested that other factors like the density of resistant bacteria, duration of exposure to the antibiotic and presence of a favourable environment need to be considered as well for developing the conditions[9,12].

The earlier studies showed presence of antibiotics in hospital effluent in developed countries. The highlighting fact that antibiotics also enter the aquatic environment through hospital effluent in India was well studied.

Unlike the developed countries, the situation can become more problematic in low- and middle-income countries, where resource constraints might result in untreated wastewater[11,29,30].

In most of the developing countries like India not much wastewater undergoes any type of treatment and along with other pollutants, antibiotics must also be finding their way into recipient waters like rivers, lakes, reservoirs, etc.

Continuous exposure of antibiotics in sub-therapeutic concentrations over long periods of time provides ideal conditions for the transfer of resistance genes. Use of non-culture techniques is important to understand the dynamics of transfer of antibiotic resistance genes[12].

Although antibiotics have been produced and applied in large quantities for some decades, until recently the existence of these substances in the environment was rendered little attention.

Several studies have detected a number of antibiotics of different concentrations in different environmental compartments like hospital effluent, municipal waste water, effluent from sewage treatment plants, surface water and in some cases ground water[16,30,31].

Different important antibiotic groups were identified such as macrolides, tetracyclines, sulphonamides, quinolones and others as far as analytical methods are available[32].

Obviously, most of the antibiotics are not fully eliminated during the sewage purification process.

The results of various investigations using test systems indicate that a number of antibiotics and disinfectants are not biodegradable in the aquatic environment[29,33]. As most of the waste water treatment systems across the world has not equipped to remove pharmaceutical compounds, which is a point of concern. Presence of antibiotics in hospital effluents, in environment and its impact towards the development of microbial resistance needs to be highlighted and necessary steps should be taken to solve the issue. At this point further low cost and environment friendly techniques could be designed and developed for the removal of antibiotics from wastewater using methods developed to simultaneously remove resistant bacteria[34].

This may offer a solution to the problem of antibiotic resistance development in the aquatic environment. Results of our study showed the scenario of antibiotics in hospital effluents, this will be helpful in creating a basis for further scientific enquiry in large populated country like India and other countries regarding the presence of emerging contaminants like antibiotics in environment and its impact on public health[35].

The LOD have some limitations and several conceptual approaches to the subject, each providing a somewhat different definition of the limit, and consequently, the methodology used to calculate that derived from these definitions, differ between them. LOD is confused with other concepts like sensitivity. Estimates of LOD are subject to quite large random variation. Statistical determinations of LOD assume normality, which is at least questionable at low concentrations. The LOD, which characterizes the whole chemical measurement process (CMP), is mistaken with concepts that characterize only one aspect of the CMP, the detection. So, further Limit of quantification (LOQ) is necessary to calculate, evaluate and confirm the presence of antibiotics in the samples. This study also determined the same consequences thus the investigators subjected all the samples for the quantification assay to determine the MDQL of the antibiotics present.

In order to properly determine the limit of detection and limit of quantification of a method, it is necessary to know the theory behind them, to recognize the scope and limitations of any approach and be able to choose the method that better suits our CMP. The intention of this study is to review the fundamentals of detection limits determination for the purpose of achieving a better understanding of this key element in trace analysis, in particular and analytical chemistry including pharmaceutical chemistry in general; and to achieve a more scientific and less arbitrary use of this data with a view to their harmonization and avoid the confusion about them, which still prevails in the environmental chemistry and toxicology community.

CONCLUSION

The results of our study illustrated the occurrence of antibiotic residues in hospital effluents. Five antibiotics ciprofloxacin, ampicillin, enrofloxacin, oxytetracycline and trimethoprim were identified in ten hospital effluents from South India.

Hospital waste water is one of the main contributors of the antibiotic entry to the sewage system and other water systems. The waste water of the hospitals is the main carrier of pathogenic microbes, pharmaceutical compounds like antibiotics and resistance bacteria. Therefore hospitals must be equipped with proper waste water treatment plants and waste water of hospitals must be treated before goes out into municipal drainage. Finally our study does highlight the need for more extensive investigation on the occurrence of antimicrobial compounds and bacterial resistance.

REFERENCES

1. Daughton CG, Ternes TA. Pharmaceuticals and personal care products in the environment, agents of subtle change, Env Hth Persp M, 107, 1999, 907-942.
2. Lai KM, Scrimshaw MD, Lester JN, The effects of natural and synthetic steroid estrogens in relation to their environmental occurrence, Crit Rev Toxicol, 32, 2002, 113-122.
3. Jonathan PB, Katerina K, Nikolaos V, Household disposal of pharmaceuticals and perception of risk to the environment, Env Toxicol Pharmacol, 21, 2006, 301-307.
4. Abuin S, Codony R, Compa R, Granados M, Prat MD, Analysis of macrolide antibiotics in river water by solid phase extraction and liquid chromatography-mass spectrometry, J Chrom, 1114, 2006, 73-81.
5. Seifrtova M, Novakova L, Lino C, Pena A, Solich P, An overview of analytical methodologies for the determination of antibiotics in environmental waters, Anal Chim Acta, 649, 2009, 158-179.
6. Grujic S, Vasiljevic T, Lausevic M, Determination of multiple pharmaceutical classes in surface and ground waters by liquid chromatography-ion trap-tandem mass spectrometry, J Chrom, 1216, 2009, 4989-5000.
7. Larsson DG, de Pedro C, Paxeus N, Effluent from drug manufactures contains extremely high levels of pharmaceuticals, J Hazard Mater, 148, 2007, 751-755.
8. Vishal D, Ashok JT, Rakesh KK, Antibiotic and antibiotic-resistant bacteria in waters associated with a hospital in Ujjain, BMC Publ Hlth, 10, 2010, 414-418.
9. Kummerer K, Antibiotics in the aquatic environment- a review- Part I, Chemosph, 75, 2009, 417-434.
10. Duong HA, Pham NH, Nguyen HT, Hoang TT, Pham HV, Pham VC, Berg M, Giger W, Alder AC, Occurrence, fate and antibiotic resistance of fluoroquinolone antibacterials in hospital wastewaters in Hanoi, Vietnam, Chemosph, 72, 2008, 968-973.
11. Vishal D, Tamhankar AJ, Aggarwal M, Sen S, Khandal RK, Stalsby LC, Detection of antibiotics in hospital effluents in India, Curr Sci, 12, 2009, 1752-1755.
12. Jarnheimer PA, Ottoson J, Lindberg R, Stenstrom TA, Johansson M, Tysklind M, Fluoroquinolone antibiotics in a hospital sewage line; Occurrence, distribution and impact on bacterial resistance, Scand J Infect Dis, 36, 2004, 752-755.
13. Kummerer K, Henninger A, Promoting resistance by the emission of antibiotics from hospitals and households into effluents, Clin Microbiol Infect, 9, 2003, 1203-1214.
14. Willis C, Antibiotics in the food chain, their impact on the consumer, Rev Med Microbiol, 11, 2000, 153-160.
15. Ternes TA, Analytical methods for the determination of pharmaceuticals in aqueous environmental samples, Trends Anal Chem, 20, 2001, 419-434.
16. Brown KD, Kulis J, Thomson B, Timothy HC, Mawhinney BD, Occurrence of antibiotics in hospital, residential and dairy effluent, municipal wastewater and the Rio Grande in New Mexico, Sci Total Environ, 366, 2006M, 772-783.
17. Koczura R, Mokracka J, Jablonska L, Gozdecka E, Kubek M, Kaznowski A, Antimicrobial resistance of integron harboring *Escherichia coli* isolates from clinical samples, waste water treatment plant and river water, Sci Tot Environ, 414, 2012, 680-685.
18. Xi C, Zhang Y, Marrs CF, Ye W, Simon C, Foxman B, Jerome N, Prevalence of antibiotic resistance in drinking water treatment and distribution systems, Appl Environ Microbiol, 75, 2009, 5714-5718.
19. Alpay SK, Ozgumus OB, Sevim E, Kolayli F, Sevim A, Yesilgil P, Investigation of antibiotic resistance profile and TEM-type β-lactamase gene carriage of ampicillin resistant *Escherichia coli* strains isolated from drinking water, Ann Microbiol, 57, 2007, 281-288.
20. Golet EM, Alder AC, Hartmann A, Ternes TA, Giger W, Trace determination of fluoroquinolone antibacterial agents in urban wastewater by solid-phase extraction and liquid chromatography with fluorescence detection, Anal Chem, 73, 2001, 3632-3638.
21. Hirsch R, Ternes T, Haberer K, Kratz KL, Occurrence of antibiotics in the aquatic environment, Sci Tot Environ, 225, 1999, 109-118.
22. Molstad S, Lundborg CS, Karlsson AK, Cars O, Antibiotic prescription rates vary markedly between 13 European countries. Scand J Infect Dis, 34, 2002, 366-371.
23. Al-Ahmad A, Daschner FD, Kummerer K, Biodegradability of cefotiam, ciprofloxacin, meropenem, penicillin G, and sulfamethoxazole and inhibition of waste water bacteria, Arch Environ Cont Toxicol, 37, 1999, 158-163.
24. Kummerer K, Al-Ahmad A, Mersch SV, Biodegradability of some antibiotics, elimination of their genotoxicity and affection of waste water bacteria in a simple test, Chemosph, 40, 2000, 701-710.
25. Narvaez VJF, Jimenez CC, Pharmaceutical products in the environment: sources, effects and risks. Vitae, 19, 2009, 93-108.
26. Caliman FA, Gavrilescu M, Pharmaceuticals, Personal Care Products and Endocrine Disrupting Agents in the Environment- A Review, CLEAN-Soil Air Wat, 37, 2009, 277-303.

27. Gholikandi GB, Dehghanifard E, Sepehr MN, Torabian A, Moalej S, Dehnavi A, Yari AR, Asgari AR, Performance evaluation of different filter media in turbidity removal from water by application of modified qualitative index, Ir J Publ Hlth, 41, 2012, 87-93.

28. Asperger D, Mutavdzic D, Babic S, Horvat AJM, Kastelan MM, Solid-phase extraction and TLC quantification of enrofloxacin, oxytetracycline, and trimethoprim in wastewater, J Plan Chrom Mod TLC, 19, 2006, 129-134.

29. Benito PE, Partal RAI, Leon GME, Moreno BMC, Evaluation of mixed mode solid phase extraction cartridges for the pre concentration of beta-lactam antibiotics in wastewater using liquid chromatography with UV-DAD detection, Anal Chim Acta. 556, 2006M, 415-422.

30. Feitosa FJ, Temime B, Chiron S, Evaluating on-line solid-phase extraction coupled to liquid chromatography-ion trap mass spectrometry for reliable quantification and confirmation of several classes of antibiotics in urban wastewaters, J Chrom, 1164, 2007, 95-104.

31. Garcia CAM, Gamiz GL, Lara FJ, del OIM, Cruces BC, Applications of capillary electrophoresis to the determination of antibiotics in food and environmental samples, Anal Bioanal Chem, 395, 2009, 967-986.

32. Garcia GMJ, Diaz CMS, Barcelo D, Determination of 19 sulfonamides in environmental water samples by automated on-line solid-phase extraction-liquid chromatography tandem mass spectrometry (SPE-LC-MS/MS), Talanta, 81, 2010, 355-366.

33. Joshi S, Sharma A, Rawat MSM, Dhiman C, Development of conditions for rapid thin layer chromatography of β-lactam antibiotics, J Plan Chrom Mod TLC, 22, 2009, 435-437.

34. Kasprzyk HB, Dinsdale RM, Guwy AJ, The effect of signal suppression and mobile phase composition on the simultaneous analysis of multiple classes of acidic/neutral pharmaceuticals and personal care products in surface water by solid-phase extraction and ultra performance liquid chromatography-negative electrospray tandem mass spectrometry. Talanta, 74, 2008, 1299-1312.

35. Mutavdzic D, Babic S, Asperger D, Horvat AJM, Kastelan MM, Comparison of different solid-phase extraction materials for sample preparation in the analysis of veterinary drugs in water samples, J Planar Chroma Mod TLC, 19, 2006, 454-462.

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