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Review Article

ANALYSIS OF DIFFERENT THERAPEUTIC CLASSES USING LIQUID CHROMATOGRAPHY - MASS SPECTROMETRY IN AQUATIC ENVIRONMENT: A REVIEW

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ABSTRACT

Pharmaceuticals included in this review are seventeenth with different therapeutic classes: anti hypertensive (Nifedipine Prazosin HCL Chlorothiazide HCL Enalapril), non steroidal anti inflammatory drugs (Diclofenac Ibuprofen Naproxen Mefenamic acid), antibiotic (Erythromycin), antidiabetic (Metformin Gliclazide), inhabit stomach acid (Ranitidine), simultant (caffeine), harmonal contraception (Levonorgestrel), anticonvulsant (Carbamazepine), bronchodilator (salbutamol), lipid lowering agent (Simvastatin). Large quantities of pharmaceuticals are used today in human and veterinary medicine and many of these pharmaceuticals are excreted without being entirely metabolized in human or animal, so these pharmaceutical residues in aquatic environment are considered sever contaminants. Progress in instrumental analytical chemistry has resulted in the availability of methods that allow a monitoring of these contaminates at ng/L levels. In the current article, a review of the Liquid Chromatography instruments provided with tandem mass, TOF, Q-TOF, Micro Triple Mass and so on, based methods published so far for determination of pharmaceuticals in the aquatic environment is presented. This review also covers the new methodologies to analysis acidic and basic pharmaceuticals; including sample preparation and solid phase extraction are discussed.

Keywords: Pharmaceuticals, SPE-LC; Aquatic environment, Recovery, Multi-Residue.

INTRODUCTION

The pharmaceutical products play an important role in the treatment and prevention of disease in both humans and animals[1]. Pharmaceuticals are considered emerging organic contaminants, so the presence of these compounds in aquatic environment might prove to be an issue in the quality of water supplies[2]. Several hundred active compounds of pharmaceuticals are used in vertinary and human drug formula with broad applications range of pharmaceuticals so their residues can reach the environment via several routes. The main reasons to increase these pharmaceuticals in aquatic environment are urinary of faecal excretion and inefficient treatment in sewage treatment plants[3,4]. Removal percentages of lipid regulating agents were between 34% and 50%[5,6]. The occurrence of pharmaceuticals in effluents by the WWTPs, Hospital effluents waste has received increased attention in recent years [7-10]. The main source to introduce the pharmaceuticals to aquatic environment is sewage treatment effluents plant, furthermore the pharmaceuticals used in veterinary participate to pollute environment in which many pharmaceuticals introduce to soil then to water via manure or direct to the water especially when used in fish farms.

The concentration of pharmaceuticals that introduced from WWTPs in ng/L, so this trace concentration does not threat drinking water but the consequences of presence of these compounds in aquatic environment unknown completely [11-14]. There are several analytical methods were used to analysis of traces concentrations of pharmaceuticals in aquatic environment.

Table 1: Physico-chemical properties of all 17 compounds [15-19].

Name of Compound	Therapeutic Class	Log kow	PKa	Property	Water solubilit	FW	Chemical structure
Nifedipine	Antihypertensive	2	NR	Basic	Insoluble	346.34	$C_{17}H_{18}N_2O_6$
Prazosin HCL	Antihypertensive	1.3	NR	Basic	0.5mg/ml	383.40	$C_{19}H_{21}N_5O_4$
Hydrochlorothiazide HCL	Antihypertensive	-0.07	8	Basic	266mg/L	295.72	$C_7H_6ClN_3O_4S_2$
Diclofenac-Na	Non – steroidal anti – inflammatory (nsaid)	3.91	4.2	Acidic	50mg/ml salt	296.15	$C_{14}H_{11}Cl_2NO_2$
Ibuprofen	Non – steroidal anti – inflammatory (nsaid)	3.97	4.3	Acidic	0.049 mg/ml	206.29	$C_{13}H_{18}O_2$
Naproxen	Non – steroidal anti – inflammatory (nsaid)	3.18	4.15	Acidic	15.9 mg/L	230.27	$C_{14}H_{14}O_3$
Mefenamic acid	Non – steroidal anti – inflammatory (nsaid)	5.12	4.2	Acidic	20 mg/L	241.29	$C_{15}H_{13}Cl_2NO_2$
Gliclazide	Antidiabetic	2.6	NR	Basic	NR	323.41	$C_{15}H_{21}N_3O_3S$
Enalapril	Antihypertensive	0.07	3.2	Basic	0.025g/mL	376.45	$C_{20}H_{28}N_2O_5$
Metformin	Antidiabetic	-0.5	12.4	Basic	Freely soluble as salt HCL	129.16	$C_4H_{11}N_5$
Erythromycin	Antibiotic	3.06	8.9	Basic	1.44mg/L slightly	733.93	$C_{37}H_{67}NO_{13}$
Ranitidine	Inhabit stomach acid	0.27	8.2	Basic	24.7mg/ml	314.41	$C_{13}H_{22}N_4O_3S$
Levonorgestrel	Hormonal contraception	3.8	NR	Basic	2.05mg/L	312.45	$C_{21}H_{28}O_2$
Carbamazepine	Anti convulsant	2.45[a]	13.9[d]	BASIC	17.7mg/L	236.27	$C_{15}H_{12}N_2O$
Simvastatin	Lipid lowering	4.7	13.5	BASIC	0.76mg/L	418.57	$C_{25}H_{38}O_5$
caffeine	stimulant	-0.07[c]	14[d]	BASIC	22mg/ml	194.2	$C_8H_{10}N_4O_2$
salbutamol	Bronchodilator	0.11[m]	9.2[n]	Basic	3mg/L	239.31	$C_{13}H_{21}NO_3$

Different classes of pharmaceuticals were detected in waste water treatment plants effluents (WWTPs), influents, municipal wastewater and surface water using UPLC-MS/MS, UPLC-TOF and LC-MS/MS[9,20-24]. Ranitidine, erythromycin and carbamazepine have been investigated in river water using LC-ESI-TQ-MS/MS combinated with ultra performance liquid chromatography –TOF-mass spectrometry [25]. HPLC-Uv was used to analysis pharmaceuticals as tablates but with S/N ratio less than LC-MS, because of the high sensitivity for MS detector compared with Uv detector [26,27].

This paper reviews different analytical methods to analysis, 17 basic and acidic pharmaceutically active compounds with different therapeutic classes (**Table 1**) using liquid chromatography.

SAMPLE PREPARATION

There are many steps to preconcentrate the trace concentration samples. One of these steps that is more used widely in analysis of trace concentration of pharmaceuticals in water is extraction by solid sorbent. Water samples include drinking water, ground water, rivers and wastewater can be extracted directly by SPE because some of them like drinking water not heavily laden with solid particles meanwhile ground water, rivers and wastewater need to be filtered before SPE procedure. The heavily laden samples with solid particles will clog the cartridge so filtration process is important but sometimes filtration process has problem related with recovery of the analytes because some of these analytes bonded to the solid particles in water so their recoveries will decrease. Effluents and influents of sewage samples were filtered through a 0.45µm glass fiber filter (millipore) and acidified to pH 3 [28], pH2 [29, 30] based on properties of target compounds. The wastewater samples from hospital effluents were passed through 0.7 µm glass fiber filter and stored at $4C^0$ to avoid any degradation [31]. Water samples (sewage water, surface water and ground water) were filtered using 0.45µm filter the filters were prewashed with n-Hexane, acetone, methanol, milli-Q water and pH was adjusted to pH10 with 2M NaOH [32]. 2L of water samples, effluent wastewater, were stored at $4C^0$ until filtration and analysis. Before extraction samples were filtered on a glass micro-fiber filter GF/D 2.7µm [33]. 150ml or 40ml of water samples, STP effluent and river, were filtered by glass fiber filtered <1 µm [34]. All samples containers (finished drinking water, surface water, wastewater effluent and septic tank influent) contained 1g/L NaN_3 for preservation of samples and 50 mg/L ascorbic acid to quench any residual oxidant, after that all samples stored at $4C^0$ until extraction experiment except septic tank samples, were filtered using 90 mm, GF/F [35]. 1.0 L of samples, effluent of wastewater and river water, was stored in dark glass bottles and kept at $4C^0$, after that filtrated immediately through 0.45µm mixed cellulose membranes in order to remove particulate matter and colloids that might otherwise affect the extraction performance and possibly provide a base for adsorption of pharmaceuticals and microorganism, pH for all water samples were adjusted to 2 with 6 M HCl [36].

SOLID PHASE EXTRACTION

Solid phase extraction steps were pretreatment of the sample, conditioning of the cartridge, loading of the sample and elution of the analytes. In this paper we will focus on reversed –phase SPE to extract 11 basic pharmaceuticals, in which the sorbent in reversed phase SPE was derivatizated with carbon chains to retain the analytes that have low polarity. Many of pharmaceuticals whose polarity ranges from mid to low polarity so it is better to use reversed phase. The most of pharmaceuticals have hydrophobicity properties (hate to mix with water) and had different properties in neutrality, acidity and basicity therefore pH plays an important role during SPE procedure to avoid deprotonation of acidic pharmaceuticals and protonation of basic pharmaceuticals so many researchers advise to make pH approximately 2 for analysis of acidic compounds and pH approximately 10 for analysis basic pharmaceuticals and pH approximately 7 for analysis neutral compounds (**Table 2**).

Weigel noticed the recovery of acidic pharmaceuticals was not good, the reason may be attributed to extraction step (incomplete) because he used pH8.3, so may be the acidic pharmaceuticals stay in their ionic forms, while caffeine its recovery fairly good because this pH suitable to extract caffeine also there is another reason related to elution step, he used large volume (90mL) of ethyl acetate, medium polar solvent [37]. MCX Oasis was used to extract basic, acidic and neutral at low pH, it was active because it has sulfonic acid group bind with ionized basic pharmaceuticals, and the acidic as well as neutral retain on reverse phase, so drugs with amino groups like hydrochlorothiazide, salbutamol and ranitidine will be charged at pH2 [33].

Table 2: Influence of type of cartridge and steps of SPE on recovery different classes of pharmaceuticals.

Target compounds	SPE cartridge	Conditioning step	Sample loading	Washing step	Elution step	Recovery	REF
Caffeine Ibuprofen diclofenac	500 mg OASIS, HLB	5 ml n- hexane, 5 ml ethyl acetate, 10 ml methanol and 10 ml tap water.	1.0 L water sample adjusted to pH=7-8, and loaded at flow rate 15 ml / min	5 ml deionized water and dried by N2 flow.	30 ml methanol	95[a] 74 87	41
Carbamazepine Ibuprofen, Diclofenac Naproxen	Oasis HLB and Strata-X	4 ml methanol followed by 8 ml acidified water pH2 (1 ml HCL to 1.o L water)	1.0 L of effluent filtered sample 500 ml of influent filtered sewage sample, flow rate 10-15 ml/min.	2 ml of methanol – water (20% v/v) after drying for 5 min.	2 x2 ml of ethyl acetate, the combined fractions of each cartridge were passed through 6.5 g of anhydrous sodium sulfate.	90[b] 91 84 83	42
Ranitidine Erythromycin Carbamazepine Mefenamic acid Diclofenac Ibuprofen	Oasis HLB 200 mg	6mL of methanol and 6ml of distilled water at flow rate 1 ml/min.	100 ml of sample adjusted to pH7(using H2SO4, 2N) flow rate 10 ml/min.	5 mL distilled water after that cartridges were dried by N2 gas for 10 min.	2×4 ml of methanol at flow rate 1 ml/min.	44.8[c] 95.2 88.1 100.1 113.6 111.7	10
Caffeine Nifedipine	Glass cartridge filled with 500 mg of Isolute C18(Bad-Homburg)	Not reported	1.0L of filtered sample was adjusted to pH(7-7.5) with H2SO4 (3M). at 2oml/min.	Not reported	After 1.0 hour of loading the cartridges were dried and eluted three times with 1 methanol.the extracts were reduced to 20 µl using N2 gas stream and then completed to 1 ml with buffer phosphate buffer and stored at -20C.	57[d] 91	43

a: tap water spiked with concentration range 20-30ng/L. b/ 1.0 L filtered ground water acidified to pH 2 and spiked with concentration 1ppb

. c/ river water intrabatch(n=5) spiked with concentration 100 ng/L. d/ ground water (n=3) spiked with concentration 200 ng/L.

Target compound	SPE	conditioning	Sampling loading	washing	elution	Recovery		REF
Carbamazapine Erythromycin Ranitidine Simvastatine Salbutamol	Oasis MCX sorbent	2 ml of methanol and equilibrated with 2 ml of water acidified with hcooh (2%hcooh, ph 2.1) at a rate of 3 ml/min.	1.0 L of water sample filtered and acidified to pH 2.5 with HCl at rate 4 ml/min.	2 ml (2%HCOOH /H2O)at 3 ml/min. after drying all cartridge were wrapped by aluminum foil and stored in freeze.	1 ml methanol and 2 ml (5% nh4oh in meoh)at rate 1 ml/min.	107.1[a] 61.6 63.4 103.8 71.5	68[b] 73.8 44.3 40.2 88.2	39
Caffeine carbamazepine	Oasis HLB (divinylbenzene / N-vinylpyrrolidone copolymer) (200mg,6cc),waters,USA	5ml of ethy acetate, 5ml of methanol and 5 ml of LC-grade water, with flow rate 1ml/min.	100 ml of sample adgusted to 7 with H2SO4.PASSED through cartridges at a flow rate 10ml/min.	5 ml of deionised water.	Cartridges were dried by N2 gas for 10 min and finally eluted with 2x4 ml of ethyl acetate at 1 ml/min.all extracts evaporated until almostdry then reconstituted with 1 ml of ethyl acetate.	92[c] 81		31

a/ high quality water spiked with concentration 200 ng/L, b/ surface water spiked with concentration 200 ng/L.

c/ wastewater (n=3) spiked with concentration of 1 µg/L.

Target compound	SPE	Conditioning	Sampling loading	Washing	Elution	Recovery	REF
Metformin Nifedipine Simvastatine Salbutamol Levonorgestrol Gliclazide Diclofenac Na Mefenamic acid Chlorothiazide Levonorgestrol	Oasis MCX cartridge 3 ml, 60 mg and 6 ml, 150 mg	3 ml MTBE(METHYL TERTIARY BUTYL ETHYL), 3ml methanol, 3 ml ultrapure water, 3 ml ultrapure water acidified with pH 2(using formic acid)	1 L, 500 ml, 200 ml, 150 ml of water filtered sample acidified to pH 2 with 37% HCL at 1 ml/min.	3 ml ultrapure water acidified with formic acid to pH 2 and dried under vacuum for 15 min.	3x2 ml methanol, 2 ml (90:10 MTBE :MeOH), 2 ml (2% NH4OH in MeOH) and finally 2 ml (0.2 %NaOH in MeOH) finally the combined elutes were evaporated to dryness under stream N2 at 45 C^0.	43[a] 61 48 49 81 70 68 93 108 81	40
Caffeine Ibuprofen Naproxen Diclofenac	6 ml (supelco-LC-18)SPE Cartridge.	6 ml hexane,3 ml acetone,6 ml DCM, followed by 2 ml deionized water adjusted to pH 2.0 with conc. H2SO4.	1.0L of sample was loaded after filtration and pH adjusted to pH 2 at 10 ml/ min., each sample bottle was rinsed three times with 10 ml of pH 2 D.H2O and the rinses also passed through SPE cartridge.	Not reported	After extraction the SPE cartridges were dried under vacuum 2 min. the target analytes were eluted with 3 successively 3 ml a aliquots of methanol at flow rate 0.5 ml / min.	110.6[b] 63.9 112.1 99.1	44
Caffeine Diclofenac Ibuprofen	Glass cartridge packed with polymer sorbent (SDB-1,2g).I.D 45mm	Not reported	10.0 L of sample loaded at flow rate 0.5L/min.	Not reported	90 mL ethylacetate followed by 50 mL of a mixture of n-hexane /ethyl acetate (4:1v/v) for neutral fraction and subsequently 50 ml for acidic fraction	72[c] 39 42	37

a/ river water spiked with 200 µL of I.S. mixture (50 ng/mL). b/ tap water spiked with range of concentration 50-200 ng/L

. c/ 20.0 L of sea water was spiked with concentration of 5 ng/L.

Aloadiany used MCX as sorbent to extract different classes of therapeutic pharmaceuticals, most of basic pharmaceuticals were not recovered well like simvastatine, metformin and salbutamol (48%, 43% and 49% respectively) may be there is not strongly bonded between the amino group of these pharmaceuticals and sulfonic group of sorbent [38].

In some cases increasing pH leads to decreasing recovery, may be this related to acidity of most compounds, so pH7 it was better to get best recovery, except ranitidine since it has high polarity and water solubility around 24.7 mg/ml, it was better extracted at high pH because its pka 8.4 means has basicity properties [10]. Recovery can be influenced by matrix because matrix leads to ion suppression so this is one of reason to effect of matrix on recovery [30]. Sometimes solvent affects recovery, using ethyl acetate to elute acidic pharmaceuticals gave not fairly good recoveries may be attributed to still acidic compounds on their ionic forms under the given conditions or to the slightly polarity solvent [37]. The basic pharmaceuticals like metformin, nifedipine, simvastatin, salbutamol, carbamazepine, erythromycin and ranitidine need to be in acidic media in order to protonate amino groups in their structures [38,39]. Extraction of carbamazepine using OASIS HLB has been influenced by the pH of sample, so the recovery of carbamazepine was 65% at pH2 [40], while at pH 7, it was 88.1%, but it was 98% at pH 10 [10]. This is related with the protonation of carbamazepine at acidic samples, so it was not be retained fairly time on sorbent.

The effect of acidifying sample on extraction for pharmaceutically active compounds (basic, neutral and acidic) was studied by Oasis HLB, quite recoveries results were obtained to extract acidic pharmaceuticals at low pH compared with basic and neutral compounds except carbamazepine, in which, the extraction of pharmaceuticals without sample pH adjustment was more superior than sample pH adjustment because in this case all of pharmaceuticals were extracted with fairly good recoveries (Fig.1) [45].

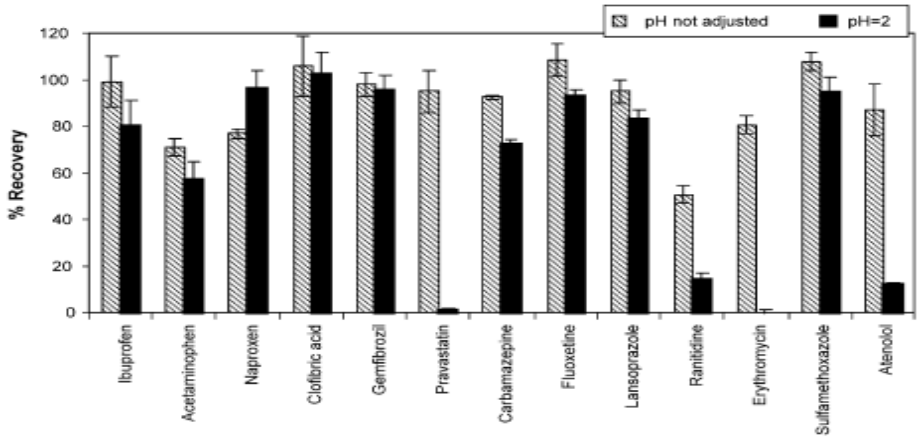

Fig. 1: Influence of pH adjustment, (pH2 and no pH adjustment), on the recoveries obtained using Oasis HLB SPE cartridges (matrix: river water and spiking 1 µg/L) adapted from Ref.(45) with permission

LIQUID CHROMATOGRAPHY - MS DISCUSSION

LC-MS is very high sensitive to detect too low concentrations in aquatic environment, but these instruments sometimes need to use isotopes standards, it is too expensive to buy. Analysis by chromatography and sophisticated MS detection techniques requires expensive instrumentation so the economic alternatives might be most beneficial for monitoring programs [25]. In liquid chromatography, no need to use derivatization reaction because all compound transferred by mobile phase, so no need to make vaporization phase of them. The increasing applications of ESI was reported in previous study, because ESI was sensitive ionizer, its function depends on high applied potential difference about 2.5-4 KV so it was preferred to connect with tandem mass and QTOF. There are two ionizer suitable to connect with LC, atmospheric pressure chemical ionization (APCI) and electro spray ionization (ESI). Where APCI differs to ESI, is in the way ionization. In ESI ionization is bought about through the potential difference (2.5-4 KV) between the spray needle and the cone along with rapid but gentle desolvation so it was soft ionization. In APCI the analyte solution is introduced into a pneumatic nebulizer and desolvated in a heated quartz tube before interacting with corona discharge creating ions. Quadrupole mass analyzer (QMS) is one type of mass analyser used in mass spectrometry, it consists 4 circular rods, set parallel to each other. It responsible to filtering sample ions based on their m/z. Ions are separated in quadrupole based on the stability of their trajectories in the oscillating electric fields that are applied to the rods, only ions with certain m/z reach to detector (without collision with rods). The development of quadrupole mass was (QQQMS) more sensitive because has a linear series of three quadrupoles. The Q1 and Q3 quadruples act as mass filter, and the middle Q2 quadrupole is employed as a collision cell. Subsequent fragments are passed through to Q3 where they may be filtered or fully scanned. This process allows for the study of fragments (daughter ions) that are crucial in structure elucidation. The most famous instrument to analysis pharmaceuticals at too low levels in water samples (rivers, lake, groundwater, WWTPs, and hospital effluents) was High Performance Liquid Chromatography connected with mass detector. Multiple classes of acidic and neutral pharmaceuticals in surface water were detected using UPLC –NET-MS [30]. LC-MS/MS was used to detect Carbamazepine in surface water, wastewater and sewage treatment plants [32]. Hilton applied HPLC-ET-MS to analysis erythromycin, ibuprofen, mefenamic acid and diclofenac in sewage effluents and surface water [28]. LC-ES-MS-MS was used to detect caffeine and nifedipine in ground water, rivers and wastewater [43] **(Table 3)**.

MCX plays an important role to extract basic pharmaceuticals at low pH because these compounds will be protonated at this pH to retain on cartridges, comparing with oasis HLB this cartridge has more efficiency to extract different pharmaceuticals in acidic, basic and neutral because this sorbent is a combination of hydrophilic – lipophylic polymer, so it can extract acidic, basic and neutral analyte at wide range of pHs [45]. LC-QTOF-MS was used to detect caffiene, carbamazapine, naproxen, ibuprofen and diclofenac in surface water close to the effluent of a sewage treatment plant (STP) and along a coastal gradient from a STP effluent [46]. Carbamazapine, enalapril, hydrochlorothiazide, ibuprofen, naproxen and ranitidine were detected in sewage treatment plant (STP) influent and effluent wastewater using HPLC/QTOF-MS [29]. LC-MS/MS was used to analysis caffiene, carbamazapine and ibuprofen in runoff from agricultural fields and septic systems within the western Lake Erie basin [47].

Table 3: Different Detectors connected with LC-MS for residue analysis of pharmaceuticals

Compound	Method of analysis	Column	Mobile phase	LOD ng/L	matrix	REF
Erythromycin Carbamazepine Simvastatine Ranitidine Salbutamol Ibuprofen Diclofenac Naproxen Mefenamic acid	LC-ESI-Micro Triple Mass	C18 column (1.7µm; 1mmx100mm)	Basic/Neutral Water, methanol and acetic acid 0.5%. Acidic/Neutral Water, methanol,0.5% acetic acid and 5mM NH4OH or 10mM TrBA.	0.1 0.1 20 1 0.1 0.1 0.05 0.1 0.1	River water	48
Caffeine Carbamazapene Naproxen Ibuprofen Diclofenac	UPLC-QTOF-MS	HSS T3(2.1×100mm,dp1.8µ,m) (waters,milford USA)	A: 10Mm acetic acid in water B: 10mM acetic acid in acetonitrile. At 0.6 ml/min.	8 1 12 7 4	Surface water.	46
Carbamazeoine Ranitidine Erythromycin Naproxen Ibuprofen Diclofenac Mefenamic acid	LC-ESI-Micro Triple Mass.	Purospher Star RP-18 endcapped column(125mmx2.0mm;particle size 5µm)and a C18 guard column.	Negative ion mode: A: methanol. B: water. Flow rate 0.2 ml/min. Possitive ion mode: A: mixture of acetonitrile – methanol (2:1). B: buffer consisting in ammonium acetate 5 Mm/ acetic acid at Ph 4.7. flow rate 0.2 ml/min.	2^S 10^E 2 20 4 6 7 9 8 12 2 10 0.5 1	Surface water and effluent wastewater	45

E: effluent wastewater, S: surface water,

Compound	Method	Column	Mobile Phase	LOD ng/L	Matrix	REF
Metformin Ranitidine Caffeine Ibuprofen	HPLC-ESI-MS	Metasil Basic 3 µm, 150 mmx 2mm,C18 analyticalcolumn.	Ammonium formate/formic acid buffer (10Mm,pH3.7)aqueous phase and acetonitrile were used to produce multi step binary elution gradient.	0.0034[a] 0.01 0.014 0.018	Surface water	49
Carbamazepine Erythromycin Diclofenac-Na Ibuprofen naproxen	LC-ESI-MS/MS.	Positive mode: 150mmx2.1mmGenesis column at flow rate 250 µL /min. Negatove mode: 150mmx2.1mmAppex column at flow rate 200 µL /min.	**Positive mode:** A: 0.015% ammonium acetate and 25% methanol in deionised water. B: acetonitrile 100 % **Negative mode:** A: ammonium acetate 10 Mm in deionised water B: acetonitrile 100%	20 70 36 23 50	River water	50
erythromycin Diclofenac Mefenamic acid ibuprofen	LC-ESI-Tandem mass	250 mmx2mmx5µm C18Luna	Water, methanol and 40 mM ammonium acetate in water, adjusted to pH 5.5 by the addition of formic acid at 200µL.	10 20 50 20	Effluent wastewater	51

a: all values are in µg/L

Compound	Method	Column	Mobile Phase	LOD ng/L	Matrix	REF
Carbamazepine Ranitidine Erythromycin Hydrochrolothaizide Ibuprofen Naproxen Diclofanac Mefenamic acid	HPLC-Z spray ESI-QqQ-MS	Purospher star RP-18 end capped column(125mmx2.0mm, particle size 5µm) and C18 guard column.	NI:A: MeOH B: Water PI:A: Mixture acetonitrile – MeOH (2:1) B: buffer solution NH4Ac 5Mm /HAc at pH 4.7 Both modes at 0.2 ml/min. flow rate.	2.2 1.4 12.4 4.5 98 79 160 5.7	Influent wastewater	52
Caffeine Carbamazepine Ranitidine Erythromycin Naproxen Diclofanac Ibuprofen	LC-ESI-QQQ-MS	Kromasil 100C18 (25X0.46cm) 5 µm particle size	A: MILLI Qwater with acetic acid Ph3. B: acetonitrile 100%.	8^I 3^E 5 2 8 2 25 15 15 7 8 2 15 7	Influent and effluent wastewater	53

I: influent wastewater; E: effluent waste water.

Compound	Method	Column	Mobile Phase	LOD ng/L	Matrix	REF
Carbamazepine Diclofenac Ibuprofen	LC-UVD. At 220 nm	LichroCART RP18 (5µm particle size, 250mmx4mm.	58% acetonitrile and 0.6 mmol/L Na2HPO4 solution(42%) at flow rate 1.0 Ml/min.	0.7[a] 0.9 0.9	River water	54
Carbamazepine Mefenamic acid Diclofenac	LC-ESI-MS/MS	Nucleodur C18 Isis HPLC column (5 µm particle size) 250mmx4.0mm.	A: 0.02M formic acid in water B: acetonitrile 100%.	5[b] 5 50	Sea water	55
Carbamazepine Naproxen Diclofenac	LC-ESI-Ion Trap-MS	Mediterranea Sea 18,C18 reversed phase column of 100mmx2.1mmid,3µm particle size.	A: 20mMammonium formate B: acetonitrile Flow rate 0.3 ml/min.	0.998[c] 0.01 0.995	Effluents wastewater	56
Carbamazepine Ranitidine Erythromycin Salbutamol Enalapril Naproxen Ibuprofen Diclofenac Mefenamic acid	LC-ESI-(QqLIT)-MS	Purospher Star RP-18 endcapped column (125mm x 2.0 mm, particle size 5 µm)provided with guard column 4 x 4, 5 µm.	Negative ionization mode: A: mixture of acetonitrile – methanol (1:1, v/v). B: HPLC water. Positive ionization mode: A: acetonitrile B: HPLC water with 0.1 % formic acid.	0.03[d] 0.02 0.01 0.02 0.01 0.84 0.12 0.09 0.74	Sediment	57

a: all values are in µg/L; b: all values are in LOQ; c : all values are in LOQ ppb ; d: all values are in ng/g.

The basic of mass spectrometry, that how we can introduce our sample to the instrument, before that sample was injected by specific syringe, now MS connected with LC. Abdel-Hamid developed a method to analysis quantitively different pharmaceuticals one of them carbamazepine, using APCI and he found the range concentrations was 100-300 ng/ml for all drugs except phenytoin (0.5-1.5 µg/ml) [58]. TOF-MS, has higher resolution than commonly used mass spectrometers and accurate mass estimation and elemental composition calculation investigate of unknown or non target compounds. He used a novel solid –phase-extraction sampler in the presence of HPLC-ESI-QTOF-MS to determine 10 compound pharmaceuticals in surface sea water, the LODs were ranged from 1.0 to 13 ng/L [46]. He used LC-ESI-ionTrap-MS to determine acidic and neutral pharmaceuticals in river and wastewater, and he evaluated the nebulizer pressure, drying gas flow –rates, drying gas temperature and capillary voltages on the intensity of LC peak for each target to set best and most stable S/N ratio from ESI-MS/Ms signals [59]. LC-ESI-Quattro Micro QQQ-MS was used to study seasonal variations in the occurrence and fate of basic and neutral pharmaceuticals in river-lake system [60]. There is a large difference in value between full scan and SIM,so the low values with SIM is good to analysis heavily polluted water samples [61].

MATRIX EFFECT

One drawback of ESI is matrix effect, because it is more susceptible to the components present in matrix, so these components lead to signal suppression or enhancement after that give erroneous results. There are several strategies to reduce matrix effect, selective extraction, effective sample cleanup after extraction and improvement of chromatographic separation. Sometimes, these approaches are not the appropriate solutions, because they could lead to analyte losses, furthermore long analysis times [45], other reasonable solution reported in previous studies [62-64], consisted in the use of suitable calibration approaches, such as external calibration using matrix –matched samples, standard addition or internal standard, as well as the dilution of sample extracts. Standard addition is a reliable method, but it is time-consuming. On the other hand, appropriate internal standard (structurally similar un labelled compound or isotopically labelled standard) are not always commercially available or they are expensive. Sometimes sample extract dilution is an effective alternative solution. The efficiencies of these three strategies have been extensively studied for each analyte in WWTP effluent and influent samples as well as the sample extract dilutions. The signal obtained after sequential dilution of WWTP effluent and influent (1:2, 1:4, and 1:8). For influent wastewaters, dilution 1:4 was required to solve this problem but this decrease the sensitivity, so this is drawback that should be taken into account. For effluent wastewaters, dilution 1:2 was enough to avoid the signal decrease for the compounds analyzed by NI and PI mode [45]. There is another solution to solve this problem, that was using flow rate reducing by post column splitting to see the enhancement on matrix effect, two flow rates were investigated 50 and 200 µL/min, matrix effects were lower at 50 µL/min compared to a flow rate of 200 µL/min in which the mean of matrix effect was reduced from 27 to 15 % by reducing the flow rate from 200 to 50 µL/min, 44% reduction in matrix effects, but for whole raw wastewater 30% reduction in matrix effect. This method may reduce matrix effect to such an extent, that external calibtarion is suitable for accurate quantification and that the standard addition procedure can be avoided. But this method had two disadvantages, it was not reliable for all analytes because some compounds did not respond at all to reduce flow rate and this method sometimes resulted in an unstable spray of ESI so peak broading and retention time shifts will be occurred [62]. In the case of river there are no effects of matrix but in the case of STP, sever suppression was noticed, loss of ion signal, at the end of chromatogram more than 10 min retention time. This is related to use acetonitrile as mobile phase,that participate to elute more hydrophobic compounds in matrix.This method involved firstly, injection standard solution, then injection extracts of matrix (river,STP influent, STP effluent), enhancement or suppression of signal was calculated by this equation:

$$[1-[(Asp-Ausp)/As]]$$

Where, As is the peak area of the analytes in pure standard solution, Asp; is the peak area in the spiked matrix extract and Ausp; is the peak area in the un-spiked matrix extract (real sample) [33]. Effect of ion suppression on recovery was investigated, he mentioned that the lower of absolute recovery of carbamazepine in STP effluent and influent samples related by ion supression of the signal during electrospray ionization (66% in influent and effluent while 98% in surface water). He calculated matrix effect for ranitidine (-28.7%), caffeine (-14.6%) and carbamazepine (-18.9%) [65].

CONCLUSION

The developed multi residue methods based on SPE following by LC-advanced spectrometric detectors / MS analysis, was successfully

applied to the analysis of hospital effluents, WWTPs effluents and influents, surface water, ground water and sea water in order to detect a wide range of pharmaceuticals with different therapeutic classes. The target pharmaceutically active compounds that were discussed in this review article, are chosen based on top 40 pharmaceutical used in Malaysia (statistic 2007 Malaysia health ministry). pH plays an important role on recoveries of target compound, meanwhile the suitable selection of cartridge is very important to improve the extraction of pharmaceuticals based on their properties. At present, a combination of LC- advanced spectrometric detectors /MS techniques appears to be the best approach to multi-compound class analysis because the application of the two complementary methodologies increases the range of compound properties that can be measured reliably. Also contribution to the analytical challenge is the trace levels (nanogram per Litter) and complex water matrices in which these pharmaceuticals are typically present in the water environment. These analytical difficulties can be reduced by optimizing sample clean-up to minimize the co-extraction and co- elution of interferences and maximize target analyte recovery. Matrix effect is challenge problem to get good results, so the good solutions was available to decrease this dramatic issue especially when the researcher deals with high contaminated samples like WWTPs and effluents from hospitals.

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