Development of method to quantify bioactive pollutants due to wastewater irrigation in environmental matrices

Garduno Jımenez A. L.[1], Parthasarathy S.[2], Ortorı C., Barrett D. A.[2], And Gomes R. L.[4]

[1]BECT – Bioprocess, Environmental & Chemical Technologies Research Group. Faculty of Engineering, University of Nottingham, NG7 2RD, United Kingdom.

[2] School of Pharmacy, Faculty of Science, University Park, University of Nottingham, NG7 2RD, United Kingdom

*corresponding author:

e-mail: Andrea.GardunoJimenez@nottingham.ac.uk

Abstract Wastewater has been shown to be the main point source of bioactive pollutants into the aquatic environment. Irrigation water comes from surface water and or re-used wastewater (treated or raw, depending on the country) meaning that the ubiquitous presence of bioactive pollutants in aquatic environments is of concern in the food growing industry. This work presents a method to analyse several matrices, namely wastewater influent and effluent, soils and plants to be able to determine the prevalence, fate and remediation of 35 of these pollutants in the context of wastewater re-use for agriculture. The extraction step varies depending on the matrix e.g. (Solid Phase Extraction and Ultra sonication) and the quantification is done by LC-MS/MS. Several parameters were studied such as pH of extraction and additives such as Na_2EDTA to improve method metrics. Most analytes presented recoveries higher than 60%, with the exception of a few such as the sulphonamides. However internal standards were used to account for matrix effects and accurately quantify recoveries. As part of the validation steps the method was tested on wastewater effluent were most of the analytes were quantified in the ng/L range, with pollutants such as tramadol, erythromycin and carbamazepine in the µg/L range.

Keywords: bioactive pollutants, wastewater, irrigation, crops, analytics

1. Introduction

Wastewater treatment plants are the main point source of bioactive pollutants (also refrred to as emerging pollutants) into the environment. Bioactuive pollutants include pharmaceuticals, natural and synthetic hormones and personal care products (Petrie *et al*., 2015). This is due to the fact that wastewtaer treatment plants were never designed to remove these pollutants. This has been shown to be a problem in areas such as the development of antibiotic resistance (Marti, E., *et al*. 2014) and endocrine disrupting effects (Burkhardt-Holm, 2010). However there are still many gaps in our understanding of their behaviour in the environment.

Alongside this water quality problem it is necessary to consider the issue of water availability. For the past five years, the World Economic Forum has ranked Water Crisis as a top five risk among issues such as Food shortage and Failure of climate change mitigation and adaption (World Economic Forum, 2017). Farming accounts for 70% of the world water use (United Nations World Water Assessment Programme, 2016) therefore irrigation for agriculture needs to be considered from a water quantity, as well as quality pespective.

Reusing wastewater for agriculture is a practice that is not only beneficial but many times necessary when there is low water availability. Furthermore it brings the added benefits of fertilizer reduction due to the high nutrient content of wastewater and the economic advantage for farmers who are reusing a waste stream to grow food (Jimenez, 2006).This practice is increasing in many countries, for example Oman (Al-Khamisi *et al*., 2016), Israel, Australia and Spain (Jimenez and Asano, 2008). In several countries, such as Mexico (Duran-Alvarez *et al.*, 2009) and Pakistan (Uzma *et al*., 2016), raw wastewater is used for irrigation without any prior treatment. When wastewtaer is reused, both after treatment and when used raw, it presents a problem in that bioactive pollutants are entering our food chain and their behaviour in environmental matrices such as soil and plants is not fully understood.

Studies have shown that bioactive pollutants are transferred from wastewater irrigation and biosolids application to land to agricultural soils and corps (Calderón-Preciado *et al*, 2009; Duran-Alvarez *et al*., 2012 Wu *et al*., 2014 Riemenschneider, 2016). However most investigation is done in laboratory or greenhouse conditions and while this is effective for careful variable control, it does not allow for the study of effects over time (such as pollutant absorption in soil).

The present work aims to develop a robust method for the analysis of bioactive pollutants in aqueous and solid (plant and soil) matrices. The pollutants analyzed for where selected to ensure coverage of the main categories of bioactive pollutants. In addition pollutants proposed for the priority hazardous substances list are included: diclofenac, 17β-estradiol (E2) and 17α-ethinylestradiol (EE2) (European Commission, 2012). Furthermore conjugates of steroid hormones are studied as it has been shown that these can undergo cleavage during the wastewater

treatment process to become the bioactive parent compound (Gomes *et al*., 2009). The list of analytes can be seen in Table 1.

This work was done with the vision of applying the method to real field conditions to advance our understanding of the introduction, fate and transformation of bioactive pollutants to the environment in the framework of wastewater reuse for irrigation.

2. Method

2.1 Extraction

Plant and soil samples were freeze dried immediately after collection to remove moisture and ensure stable storage. They were extracted by ultrasound assisted extraction (ASE) or by agitation. Aqueous samples were filtered through 0.45µm GF/C filter and extracted by SPE on the day of collection. Methanol was used as the solvent for the extractions of the solid matrices and as the eluting solvent for the SPE of aqueous matrices.

2.2 LC conditions

QTRAP LC analysis was performed on 5 µL of each sample extract injected at a flow rate of 0.3 $mLmin^{-1}$ using a Shimadzu series 10 AD VP (Columbia, USA) equipped with binary pumps, a vacuum degasser, a SIL-HTc autosampler and a column oven (Shimadzu, Columbia, USA) using a Phenomenex C18 guard Column, 150×2 mm (3 µm particle size). A gradient programme was used with the mobile phase, combining solvent A (0.1%, v/v, of formic acid in water) and solvent B (0.1%, v/v, of formic acid in ACN) as follows for positive mode: 0% B (initial), 100% B (10 min), 100% B (12 min), 0% B (13 min). For negative mode the mobile phase containing solvent A (10mM Ammonium bicarbonate in water) and solvent B (10mM ammonium bicarbonate in ACN) was used and the gradient programme was: 15% B (initial), 100% B (10 min), 100% B (12 min), 15% B (13 min). A re-equilibration time (1 min) was performed before each injection. The column and sample temperature were maintained at 50 and 40 ◦C, respectively. Instrument control and data acquisition and evaluation were performed with the Analyst 1.4.2 software package purchased from Applied Biosystems.

2.3 Mass spectrometry conditions

MS data acquisition was performed with the ESI source operating in positive ionisation (PI) and negative ionisation (NI) mode under the time-scheduled multiple reactions. The optimisation of the MS/MS experimental conditions was performed first by infusion and afterwards by on-column injection of standard solutions of the individual compounds and a mixture solution of all of them. Identification of the precursor ions was performed in the full scan mode by recording mass spectra from m/z 100 to 1000. The resulting optimised values were as follows: capillary voltage 3.5 kV; source temperature, 350 ◦C; desolvation temperature, 450 ◦C; extractor voltage 3 V; and RF lens 0.2 V. Nitrogen was used as both the nebulizing and the desolvation gas at 630 Lh^{-1}. For operation in the MS/MS mode, argon was used as collision gas with a pressure of 2.6×10^{-3} mbar. Identification of the target analytes was accomplished by comparing the LC retention time and the MS/MS signals of the target compounds in the samples with those of standards analysed under identical conditions.

To validate the method for aqueous matrices real wastewater effluent and influent as well as river water were analyzed. Wastewater samples were taken from a wastewater treatment plant in Nottingham serving a population equivalent (PE) of approximately 650 000. Soil which was regularly irrigated with spiked water containing a known concentration of bioactive pollutants was analyzed to validate the soil extraction. The soil was irrigated with the spiked water for several weeks to allow for absorption processes to take place.

3. Results

The optimum identification and quantitation LC-MS/MS parameters determined for each analyte are presented in Table 1 below. The LOD and LOQ were determined by a signal to noise ratio of three and ten respectively, for the lowest level of spiking. They were dependent on the analyte and the matrix. For aqueous samples the LOD ranged from 0.03ng/L for Trimethoprim to 3.52 for 4-Tertoctylphenol. Recoveries were also analyte and matrix dependent as well as depending on the level of spiking, they were generally above 60%.

Results obtained for the method validation in aqueous samples in Nottingham are presented in Table 2 below and the concentrations measured were found to be consistent with those available in literature (Petrie *et al*., 2015). For the data presented in Table 2 Oasis MCX SPE cartridges were used.

4. Conclusion

An efficient and reproducible sample preparation method for the simultaneous extraction of 36 bioactive pollutants in environmental samples was developed. The single extraction step considerably simplifies sample preparation. A rapid and sensitive LC-MS/MS method for the analysis under positive and negative electrospray modes with a chromatographic run of only 15 min. The method yielded detection limits in the low ng/L range for wastewaters, thus providing a reliable and robust tool that can be used for routine analysis of multiple-class bioactive pollutants in aqueous samples. The proposed methods were successfully applied to the determination of these target compounds in environmental water samples collected in the UK, demonstrating the presence of significant amounts of many bioactive chemicals such as morphine and trimethoprim in effluent wastewaters, indicating that current WWTPs cannot efficiently remove bioactive pollutants.The method presented is robust and can be used to analyze for aqueous, soil and plant matrices allowing for the study of the fate of bioactive pollutants in the framework of wastewater reuse for irrigation. Further work

Table 1. Optimum LC-MS/MS parameters and categories of analytes

Bioactive chemical	Precursor ion (*m/z*)	MRM 1	DP	CE	MRM 2	DP	CE	Rt (min)
Analgesics								
Acetaminophen	152.142 $^{+}$	135.1	31	9	110	31	23	5.51
Tramadol	264.307 $^{+}$	246.2	66	17	247.3	66	11	5.31
Anti-inflammatories								
Diclofenac	294.84^{-}	251	50	16	215	50	30	5.86
Ibuprofen	204.98^{-}	158.8	70	10	160.9	70	12	5.57
Ketoprofen	249.113^{-}	121	45	12	126.8	45	10	4.94
Naproxen	229.097^{-}	169	35	42	170	35	22	4.74
Phenazone	189.188^{+}	104.1	81	35	106	81	39	6.53
Salicylic acid	136.935^{-}	93	40	22	65.1	40	38	1.77
Beta-blocker								
Atenolol	267.146^{+}	145.1	81	39	190.1	81	27	4.94
Metoprolol	268.219^{+}	116.1	131	27	133.1	131	37	5.30
Propranolol	260.201^{+}	116.1	71	27	183.1	71	27	5.91
Compounds with estrogenic effects								
4-Tertoctylphenol	205.054^{-}	132.9	80	30	117.8	80	82	10.64
*Estradiol (*E2)	271.135^{-}	145	175	54	182.9	175	52	8.15
*Estradiol-3-sulfate (*E2-3S)	351.142^{-}	271.3	110	50	144.8	110	76	5.56
Estriol (E3)	287.088 $^{-}$	170.9	130	52	145	130	54	6.20
Estriol-3-sulfate (E3-3S)	389.158^{-}	193.9	30	18	71	30	62	4.32
Estrone (E1)	269.095^{-}	144.9	110	52	142.9	110	76	8.57
Estrone-3-sulfate (E1-3S)	371.211 -$^{-}$	267.1	120	38	349.1	120	16	5.84
*17α-Ethinyl Estradiol (*EE2)	295.17^{-}	142.9	30	74	144.8	30	56	8.44
Levonorgestral	313.246^{+}	109.1	81	41	91	81	25	9.86
Lipid-modifying agent								
Bezafibrate	361.132^{-}	275.1	65	24	274.6	65	26	5.36
Clofibric acid	214.03^{-}	127.9	30	20	126.9	30	20	4.49
Gemfibrozil	249.113^{-}	121	60	18	126.8	60	14	6.23
Simvastatin	419.3^{+}	199.1	91	17	285.2	91	17	11.32
Macrolide antibiotics								
Erythromycin	734.58^{+}	158.2	36	43	576.4	36	29	6.29
Codeine	300.201 $^{+}$	152.1	106	91	115.1	106	91	4.96
Morphine	286.129 $^{+}$	152.1	91	79	201.1	91	37	4.96
Morphine-6- glucuronide	462.192 $^{+}$	286.1	101	43	201.2	101	59	1.93
Psychiatric drugs								
Carbamazepine	237.153^{+}	194.1	136	29	193.2	136	47	8.13
Paroxetine	330.261^{+}	192.2	101	31	109.2	101	87	6.43
Sulphonamide antibiotics								
Sulphamethaxazole	254.102^{+}	156.1	66	25	108.1	66	35	7.23
Sulphapyridine	250.134 $^{+}$	156.1	56	25	108.1	56	37	6.05
Sulphadiazine	251.113 $^{+}$	156.1	56	23	108	56	37	5.90
Tetracyclines								
Oxytetracycline	497.203^{+}	98.9	71	59	480.1	71	25	8.14
X-ray contrast medium								
Amidotrizoic acid	614.759^{+}	361	96	25	233.1	96	51	7.57
Other antibiotics								

Trimethoprim	291.184 [+]	231	111	33	261.9	111	33	5.06

Table 2: Analytical method validation and performance data in aqueous matrices

Bioactive chemicals	Correlation Coefficient R^2	Low level spiking 50 (ng/L)		Intermediate level spiking 200 (ng/L)		High level spiking 750 (ng/L)		Bioactive pollutants concentrations (ng/L) in effluent wastewater from this study
		% Recovery	%R.S.D	% Recovery	%R.S.D	% Recovery	%R.S.D	
Morphine	0.9988	94.20	9.95	110.04	6.90	82.51	12.93	217.77 ±10.96
Trimethoprim	0.9958	26.73	11.64	21.65	6.58	35.96	6.69	2450.26 ±272.91
Tramadol	0.9990	11.19	8.65	9.08	10.97	14.20	3.27	125396.10 ±25475.99
Sulphapyridine	0.9993	8.50	14.65	8.99	8.97	14.67	6.15	1461.79 ±50.02
Sulphamethaxazole	0.9995	7.64	13.83	5.57	9.40	6.12	11.83	79.55 ±2.39
Propranolol	0.9988	18.32	8.09	19.90	9.10	29.82	9.30	282.76 ±14.61
Phenazone	0.9973	51.09	2.04	47.34	0.36	48.22	8.23	bld
Paroxetine	0.9995	12.62	8.69	11.99	2.78	13.39	14.10	bld
Morphine-6-glucuronide	0.9969	28.42	10.08	17.92	1.43	35.02	5.76	bld
Levonorgestral	0.9988	9.81	5.67	6.32	5.09	20.38	2.68	bld
Codeine	0.9985	7.03	12.02	6.49	12.90	12.39	5.62	1987.20 ±271.70
Acetaminophen	0.9955	12.08	13.00	12.94	7.68	25.08	3.35	35.52 ±3.90
Sulphadiazine	0.9985	11.67	14.21	12.58	10.63	22.15	4.58	32.31 ±2.91
Atenolol	0.9993	55.59	12.19	47.53	5.60	49.97	7.93	654.62 ±125.22
Metoprolol	0.9993	51.85	2.70	50.53	7.73	59.13	14.34	21.63 ±6.76
Carbamazepine	0.9957	4.37	14.94	4.13	13.86	12.69	14.61	3060.12 ±498.00
Erythromycin	0.9980	112.06	5.40	98.34	1.82	82.59	10.97	4796.07 ±3887.32
Simvastatin	0.9995	7.53	6.61	2.48	4.26	2.89	3.21	256.60 ±80.60
Amidotrizoic acid	0.9994	15.48	8.00	7.46	12.95	2.31	4.44	113.14 ±22.33
Oxytetracycline	0.9996	21.91	7.93	24.27	15.26	27.36	1.53	12.04 ±6.15
4-Tertoctylphenol	0.9995	66.31	7.70	60.68	9.97	56.27	10.27	16.74 ±3.43
*Estradiol (*E2)	0.9993	37.62	6.87	24.18	2.61	17.25	2.83	31.88 ±9.06
*Estradiol-3-sulfate (*E2-3S)	0.9994	39.25	4.83	36.72	1.84	27.43	2.54	bld
*Ethinyl Estradiol (*EE2)	0.9991	83.66	9.23	89.21	7.20	66.89	12.71	3.40 ±0.38
Ibuprofen	0.9985	59.88	9.77	78.73	9.03	63.81	11.70	20.05 ±15.67
Diclofenac	0.9990	43.46	6.74	62.13	3.60	38.25	12.22	5473.80 ±2240.80
Naproxen	0.9999	99.72	3.13	96.46	5.03	84.80	4.07	29.81 ±3.30
Clofibric acid	0.9996	79.43	8.56	77.45	8.41	74.13	10.08	2.12 ±0.07
Estrone (E1)	0.9999	59.48	12.14	74.06	10.55	66.21	14.47	22.64 ±5.30
Estriol (E3)	0.9997	76.09	6.06	67.48	5.93	62.26	2.83	1.36 ±0.06
Estrone-3-sulfate (E1-3S)	0.9989	38.74	2.47	38.56	1.42	25.92	2.24	bld
Bezafibrate	0.9991	81.40	7.05	88.74	7.35	73.69	13.55	113.21 ±10.65
Gemfibrozil	0.9994	80.74	2.01	82.32	1.49	68.47	9.30	3.12 ±0.56
Ketoprofen	0.9992	70.25	7.47	65.86	4.52	61.89	7.30	26.51 ±6.60
Salicylic acid	0.9998	104.37	3.72	101.69	1.05	108.09	7.81	97.09 ±10.32
Estriol-3-sulfate (E3-3S)	0.9990	16.97	4.87	17.79	6.39	15.03	8.49	bld

*bld: Below limit of detection

must include field investigations in areas that have been irrigated with wastewater for considerable periods of time to study long term effects of this practice

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